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April 23-26, 1978

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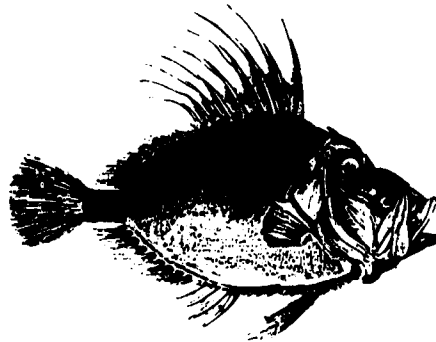
Compiled by

Ranzell Nickelson II

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FISH FLAKES OFFER NEW POSSIBILITIES FOR FINFISH UTILIZATION

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INTRODUCTION

To Charles Francatelli, master of haute cuisine and chief cook to Queen Victoria, boiled turbot meat served in a delicate sauce was a dish fit for the royal table.

According to his recipe for turbot a la creme au gratin, the cook is to boil a turbot and drain it. Then, "With a spoon, cut the turbot into flakes and put them in the sauce, taking care to waste none of the delicate meaty part of the fins, the cheeks, and the glutinous membranes of the fish" (2).

Today, researchers for the Gulf and Atlantic Fisheries Development Foundation, Inc., of Tampa, Fla., are trying to develop ways of producing fish flakes so that, before long, modern-day Francatelli's will be able to purchase them at the corner grocery.

During the 30-month project, the researchers will seek to produce the most appetizing, skin-and-bone-free flesh of various species of finfish, either alone or in combination. Moisture content will be about 65 percent and protein will be about 20 percent.

In the 1940's, Jarvis' classic text on seafood canning described a method for producing fish flakes (4). In addition, tuna processing and canning technology provides guidance for the current fish flake effort. National Marine Fisheries Service statistics for 1975 (8) show that the U. S. canned tuna pack, produced from both domestic and imported raw, frozen tuna, totaled 26.8 million cases valued at $652.6 million--an impressive example of effective utilization and marketing.

Similarities between possible methods of fish flake production and tuna processing are apparent in Gillies' book on fish and shellfish processing (3), which includes a chapter "Tuna and Tuna-like Fish."

Fish flakes from frozen cod, haddock, flounder, turbot and other species are being widely used in prepared frozen seafood products, as one can readily determine by reading labels in the supermarkets. Nevertheless, there appears to have been little research directed at fish flake manufacture in recent years. The lack of research is indicated by examining the extensive number of other products discussed at the Technical Conference on Fishery Products held in Tokyo in December of 1973 (5).

Instead, much effort has gone into mechanical deboning of fish meats, producing raw products described as "comminuted" or "minced." Machines for this purpose use pressure to force meats through openings of about five millimeters or less, separating out the pressure-resistant bones, skin and scales. Such methods fit into large-scale operations when supported by sufficient mechanization, and there are many uses for meats so produced.

However, the flaking process has some distinct advantages in producing bland meats from some rather strong-tasting species. Many land-locked Americans who dislike mullet and bluefish will find the derived flakes very acceptable.

Members of the Gulf and Atlantic Fisheries Development Foundation consider flaking to be one of the more promising approaches to marketing certain underutilized species. North Carolina was chosen as a test area because of the varied finfish in its vast estuarine and offshore fisheries. In addition, since 1965, several technological studies have been made involving preservation of the catch, methods of expanding small plants to diversify handling and processing, and development of new seafood products (1, 6, 7).

The research shows that the North Carolina crab picking plants produce products worth about $12 million. In addition, Carteret County has highly-mechanized scallop plants where production has fluctuated from near zero to millions of dollars' worth as scallop supplies have varied.

The investigators believe that initial production and utilization of fish flakes will be most effectively handled through small plants. By processing frozen raw materials into fish flakes when crabs, scallops and other fish are scarce, the plants could operate year-around. Therefore, the first objective is to develop a simple technique for producing fish flakes in such facilities, and to have a limited number of further-processed products tried on the open market.

The second objective is to mechanize fish flake manufacturing techniques, thereby making it economically feasible to undertake large-scale manufacture and to expand fish flake uses. Such expansion will require extensive marketing efforts.

MATERIALS

So far, raw materials examined for producing fish flakes have been those available in North Carolina. This limitation of potential

species is merely a matter of convenience in working out preliminaries of handling and dressing. The economic potential of fish flakes will be judged in terms of all available resources so comparisons outside the state will be made.

Comparing North Carolina prices for underutilized fish and prices for the more popular species illustrates the potential for fish flake development. In 1977, the state's catch of fish and shellfish totaled 244,750,585 pounds with an ex-vessel value of $28,374,435 (9).

Of that total, croaker made up 18,994,577 pounds valued at $2,076,370 or 10.9 cents a pound. In 1976, croaker was valued at 10.5 cents a pound.

The 1977 bluefish harvest totaled 2,331,446 pounds at an ex-vessel price of $218,663 or 9.4 cents a pound. In 1976 bluefish brought an average of 9.5 cents a pound.

Mullet totaled 1,834,935 pounds at $193,291 or 10.5 cents a pound in 1977. The 1976 price was 10 cents a pound.

In 1977, 8,671,210 pounds of gray trout brought $1,048,664 or 12.1 cents a pound. The 1976 price was 11 cents a pound.

Comparative values for the more popular flounder and striped bass are striking. In 1977, flounder brought an ex-vessel price of 44.9 cents per pound, compared to 35.4 cents in 1976. Striped bass jumped a remarkable 20 cents per pound, from 50.4 cents in 1976 to 70.8 cents in 1977.

Clearly, the prices for underutilized species like croaker, mullet, bluefish and gray trout are not keeping up with the increasing demand for seafood, as indicated by the dramatic price rise of the two more popular species. Successful production of fish flakes from these underutilized species may reverse that trend.

METHOD

PRESERVATION--Fish will be brought in aboard iced, insulated trawlers, bulk frozen in the round, and stored following North Carolina commercial practice (7).

THAWING--Frozen fish will be immersed in cold water to soften them just enough for gutting, or for cutting fillets when larger fish are used.

SKINNING--The most promising approach so far is brief immersion of partially frozen, dressed fish in boiling water, slipping off the skin and scales and washing off subcutaneous fats.

PROCESSING--Fish will be placed on trays, exposed to steam at 15 p.s.i. for time periods sufficient for thorough cooking. Meat will be separated manually from the bones and the detached bones will be removed, based on prior knowledge of locations derived from

X-ray studies. Flakes will be classified by color and other characteristics as required by intended use.

UTILIZATION--Formulation experiments will be conducted in the laboratory, then participating companies will be assisted in preparing fish flakes on a pilot plant scale. The researchers will assist companies to follow up with consumer evaluations and test marketing.

ORGANIZATIONAL DETAILS--The North Carolina Fisheries Association, which is conducting the Foundation's fish flake project, appointe Marine Chemurgics to carry out research and development. At the same time, it established a review committee consisting of representatives from processing firms which might become directly involved in pursuing project development. Progress is reported to the committee each month.

Even more direct is the involvement of two sub-committees, Pilot Plant Production of Fish Flakes and Commercial Utilization of Fish Flakes. Membership on these committees indicates willingness to provide space and facilities on plant premises for semi-commercial experiments which include processing, further processing, freezing or canning. The arrangement makes six plants available for experimental work.

RESULTS

Although still in its initial phases, the project has developed some new ideas for speeding up the hand removal of fish flakes from skin and bones. This is being achieved by careful examination of body structure of candidate species, along with selection of butchering and cutting methods which facilitate later removal of the meats.

The basic, steaming, cooling and flake separation method works equally well with small and large fish. In the course of such steaming, there is run-off aqueous oil portions which can be collected for processing into other products.

The flakes can be separated in a variety of forms depending on the end use. The aqueous portions can be separated by gravity or centrifuge to obtain a gelatinous product which will enhance the flavor if added back into certain formulations.

The flakes can be vacuum-heated in boil-in bags, frozen, then thawed for later use. Exact textural changes have not been determined, but consumer panels could not detect a change in palatability as a result of freezing. Thawed flakes have also been tried with good results in a number of products which were then frozen and subsequently baked or fried directly from the freezer.

DISCUSSION AND CONCLUSIONS

This project in its initial phases has served to determine the problems which must be solved in producing fish flakes. A major consideration is to select species which are available at low prices and can be processed economically enough to compare well with cod,

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haddock and other flakes presently being produced from frozen fillets or blocks.

It appears that properly frozen and protectively-packaged whole fish will be suitable for preparing fish flakes, since no appreciable difference has been noticed by those comparing flakes made from fresh fish and flakes from frozen fish.

The researchers have noted that the method of butchering affects the labor requirement in subsequent fish flake manufacture. Much work remains to be done to establish specific relationships between butchering methods and labor requirements for fish flake production.

The methods of separating flakes from the bones and the effects those methods have on quality requires additional study. A primary goal of the project is to state requirements for the process in specific terms.

In conclusion, there is already evidence that well-prepared fish flakes are capable of improving other seafoods when used in pre-cooked products. The investigators are increasingly disposed to insist that fish flakes can become valued ingredients for high-quality products and should not be considered "extenders."

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"SEA DOG" MADE WITH MINCED MULLET AND TEXTURED SOY FLOUR

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Underutilized fish species are a potential source of inexpensive protein. Fish protein content varies with the species and the season from 17-24% on a total weight basis. Fish proteins have high biological value and a favorable amino acid distribution for human requirements (8).

Annual harvests of the black mullet species (Mugil cephalus) are approximately 30 million pounds in Florida (17). Utilization of mullet has been limited, however, primarily because of the rapid on-set of rancidity during storage (6). The species also has a strong flavor which is objectionable to many people. Other drawbacks include a color problem and a low flesh yield (3).

The "Sea Dog", a sausage product, was designed to overcome these limitations of the mullet species. Rancidity development can be controlled by adding a liquid smoke flavoring which has been demonstrated to have antioxidant properties (7,18). The distinctive flavor characteristics of mullet can be masked by the inclusion of the relatively high spice levels typical to sausage products. The presence of both dark and white flesh in mullet has prevented its use in popular seafood products such as fish sticks (1), but is not detrimental to a sausage product because visible particles are typical to coarse ground sausages (12). The flesh yield can be increased by the use of mechanical deboning machines which can increase yields by an additional 10-15% (2,5). An added bonus of a sausage product is consumer convenience, an important marketing consideration in the U.S. (11). The sausage manufacture requires no special equipment, particularly important in Florida where most seafood dealers are small businessmen who can not afford large capital expenses.

Extensive research has been done on fish sausages in Japan, but these products contain up to 10% starch which imparts a rubbery texture unsuitable for American consumers. U.S. researchers have developed fish sausages but with added oil (2,10). The sausage studied here differs from the previous products in that it contains neither added starch nor fat. Another distinguishing feature is the addition of up to 30% (by weight) of rehydrated textured soy flour as an extender to decrease manufacturing costs.

The purpose of this study was to examine the effect of three factors (soy, water, and sodium tripolyphosphate) on the sensory acceptability of the sausage product. Textured soy flour was included because it can contribute nutritional, economic, and functional advantages (15). Sodium tripolyphosphate (TPP) has been demonstrated to increase cooking yields as well as to improve sensory characteristics

of fish products (14). Water was studied because of its effect on the textural properties of the product (13).

MATERIALS AND METHODS

Sample preparation

Mullet were purchased fresh for each experiment from Cedar Key, Florida and transported to Gainesville where they were filleted, skinned, washed, and stored on ice in a 35°F cooler before use. Sausages were always prepared on the day of fish purchase to assure uniform freshness. The chilled fillets were chopped and ground to simulate deboned flesh, and mixed with 100ppm sodium nitrite, 1.6% sodium chloride, 0.9% sugar, 1.1% spices, and 0.5% liquid smoke flavoring (Charsol C-6, Red Arrow Co.) for all samples. (All percentages were calculated using total raw weight as a basis and were chosen from formulations used in preliminary studies). Sausages were prepared using 0-30% textured soy flour, rehydrated as 1 part soy to 3 parts water (Centex 400, caramel, Central Soya), 0-30% water (as ice), and 0-0.6% sodium tripolyphosphate (granular food grade, FMI). All ingredients were stirred 50 strokes by hand in a mixing bowl and ground using a home electric food grinder (Hamilton Beach Co., Model 233) using a coarse plate. The ground batter was thoroughly mixed, reground, and stuffed into edible collagen casings (TeePak Coria®, 30mm diameter). Sausages were baked at 375°F for 50 minutes in an electric oven on a rack to allow for drainage of cooking juices. Sausages were cooled for 30 minutes, weighed, and stored at 35°F in barrier bags (Cryovac®) to prevent moisture loss.

Subjective evaluation

Sensory panels were always held the day following sample preparation, and samples were evaluated by the same nine experienced judges. Sausage samples were heated for 20 minutes at 400°F before evaluation. Samples were sliced, numerically coded, and served in random order to judges in individual booths with red lighting to mask color differences. Three experimental combinations and a reference (containing 20% soy, 10% water, and no tripolyphosphate) were included in each panel session. The categories judged were flavor, texture, and overall acceptability using a seven point hedonic scale which assigned 1 to extremely poorer than reference, 4 to same as reference, and 7 to extremely better than reference.

Objective evaluation

Percent cooking loss (as an indicator of product texture) was calculated in triplicate using the weight difference between raw and cooked samples, divided by the raw weight and multiplied by 100. Shear force values were obtained with a Texture Test System® Model TP1 (Food Technology Corporation) using a 136 kg ring and a maximum descent speed of 0.61 cm/sec with a standard blade shear compression cell. Core samples were cut to 2x2 cm cylinders and data are reported as direct readings in pounds for 4 to 8 replicates. Expressible water was

measured using the procedure reported by Hamm (9) with a larger
sample size to accommodate the non-homogeneous samples. to assure
uniform freshness, shear force and expressible water were measured on
the same day as the sensory evaluation.

Statistical analysis

Response surface methodology was applied to the data. One major
advantage of this type of analysis is that it allows for predictions
of responses for factor combinations not included in the actual exper-
iment. In addition, response surface analysis provides a complete
summary of the experimental results, including trends, factor inter-
actions, and multiple optimum combinations (if they exist). The quad-
ratic polynomial model shown below was used for analyzing the data (b
represents a beta value) for linear, quadratic, and interaction effects.

$$\hat{Y} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 +$$
$$b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Contours of a constant response were calculated using a computer by
setting one factor as a constant and solving the equation for various
combinations of the other two factors. The effect of the third factor
was estimated by changing the value of the fixed factor and solving
the equation at the new level.

Five levels of each factor (shown in Table 1) were chosen accord-
ing to a central composite rotatable design configuration for three
factors (4). Seventeen combinations, including three replicates of
the center point, were tested in random order.

RESULTS AND DISCUSSION

Regression analysis

Analysis of variance was calculated for all six responses along
with the coefficient of determination, a measure of how well the sta-
tistical model fits the data. Possible R^2 values range from 0 to 1,
with 0 indicating no fit and 1 indicating perfect fit. In this study,
only R^2 values greater than 0.900 were considered accurate enough for
prediction purposes.

Low R^2 values were found for expressible water (0.756), flavor
panel scores (0.367) and overall acceptability scores (0.790). A lack-
of-fit test was highly significant ($\alpha < 0.01$) for expressible water,
implying that a higher degree model may be required to explain this
response. A lack-of-fit test for flavor panel scores was not signifi-
cant, and the extremely low R^2 indicates that the three factors consid-
ered did not affect flavor. This result was expected because neither
water nor tripolyphosphate (TPP) at the levels used should affect the
flavor. This category was evaluated to confirm the prediction that
even high soy levels are not detrimental to flavor. The lack-of-fit
test was almost significant ($\alpha = 0.084$) for overall acceptability scores.

The model was therefore approximate, but not accurate enough for prediction purposes. No further statistical analysis was performed on these three responses because of poor model fit.

The R^2 values for texture panel scores (0.900), shear force (0.935), and cooking losses (0.923) were all high and predictions could be made at a confidence level greater than 0.01.

Regression analysis showed that the water level significantly affected the shear force, cooking loss, texture panel, and overall acceptability scores ($\alpha < 0.01$). Tripolyphosphate significantly influenced the shear force values and cooking losses ($\alpha < 0.01$) while the two categories in the sensory analysis were significantly affected by the soy level ($\alpha < 0.05$).

Texture panel scores

Fig. 1 shows the effect of tripolyphosphate (TPP) and H_2O on the texture panel scores at three fixed soy levels. The contour lines around the center point (5.0) represent the estimated panel responses. For example, any point on a 4.8 contour represents a combination of TPP and H_2O which will give a texture panel score of 4.8. One important observation is that quite different combinations of TPP and H_2O can give the identical texture panel score of 4.8. Specifically, when no soy is used in the sausage formulation (Fig. 1-A), the combinations of 0.1% TPP with 8% H_2O, 0.1% TPP with 26% H_2O, and 0.3% TPP with 15% H_2O will yield the same value (4.8) for the texture panel score.

A general trend observed with increasing soy levels was a shift of the contours downward and toward the right, showing that different combinations of TPP and H_2O are required to achieve the same optimum value (5.0) at different soy levels. At 30% soy, (Fig. 1-C) a maximum response could not be attained because the center point had shifted outside of the experimental range. Twenty-five percent soy was the maximum level allowed for a maximum texture panel score of 5.0.

The general trends for TPP and H_2O are similar for the various soy levels in Fig. 1. As the level of either factor increased, (at a given soy level) the texture panel score increased to a maximum and then decreased. Both factors have a tenderizing effect on sausage texture (12,16), so initially the product was too tough while at high levels the product was too tender. This resulted in a lower score as defined in the panel scale which assigned lower scores to samples less desirable than the reference regardless of the cause.

Shear force values

Contours for the shear force values are shown in Fig. 2 for three fixed water levels. In comparing these shear force values to the previous texture panel scores in Fig. 1, a correlation must be made, remembering that shear force values increase as the product becomes tougher (a linear response) while texture panel scores increase to a maximum and then decrease (a quadratic response). Preliminary studies showed an optimum (not maximum) value of approximately 19 lb for shear

force. Again, many different factor combinations can give an optimum response of 19 lb at a fixed water level.

A general trend with increasing water level was a shifting of the contours to the right, indicating increased tenderness as observed before in Fig. 1. At 30% water (Fig. 2-C) an optimum response could not be attained, and the maximum water level for maximal acceptability was approximately 25%. General trends for the effect of soy and TPP vary with the water level. At water levels above 10% (Fig. 2-B and 2-C), optimum texture was achieved only with high soy levels combined with low TPP levels. TPP had only a tenderizing effect, and soy had only a toughening effect. At water levels less than 10%, specific factor combinations varied from the general trends. For example, at 0% water (Fig. 2-A), two optimum regions occurred--one with high soy and moderate TPP levels, and one with low soy and moderate TPP levels. Low soy levels (0-10%) had a tenderizing effect, while high soy levels (10-30%) had a toughening effect. Furthermore, soy had a consistently tenderizing effect when combined with levels of TPP greater than 0.45%. TPP had the expected tenderizing effect at soy levels greater than 10% but could actually toughen the product at low soy levels. This interaction between soy and TPP can not be explained by current theories of TPP action.

Cooking losses

Fig. 3 shows response surface contours for cooking losses at three fixed soy levels. The general trend for increased water addition was increased cooking losses. Net retention of water was greater at higher water levels, however, so the effect of added water was still tenderizing. Increasing levels of TPP caused a decrease then a slight increase in cooking losses, and for all five levels of soy ranging from 0-30%, the transition point varied from only 0.37-0.45% TPP. This implies that TPP levels greater than 0.45% can actually increase cooking losses, a surprising finding since TPP was expected to consistently increase cooking yield.

To determine the accuracy of the predicted values, the actual observed values for the reference sample were compared to predicted values obtained using response surface methodology. The differences between the observed and predicted values were small (0.12 for texture panel scores, 1.25 lb for shear force, and 0.37% for cooking loss), demonstrating the usefulness of this methodology in a practical application.

CONCLUSIONS

Response surface methodology was successfully applied to both subjective and objective data. Accurate predictions could be made for texture panel scores, shear force values, and cooking losses. Water and TPP had a general tenderizing effect while soy had a toughening effect. Variations from these general trends, however, were observed for specific factor combinations. Multiple optimum combinations were possible for the product, so a particular formulation could be chosen for economic considerations to lower production expenses.

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Table 1. Five Levels of Three Factors Used for Response Surface Analysis.

Factor	Percentage Levels				
Soy	0	6.08	15	23.92	30
Water	0	6.08	15	23.92	30
TPP	0	0.122	0.3	0.478	0.6
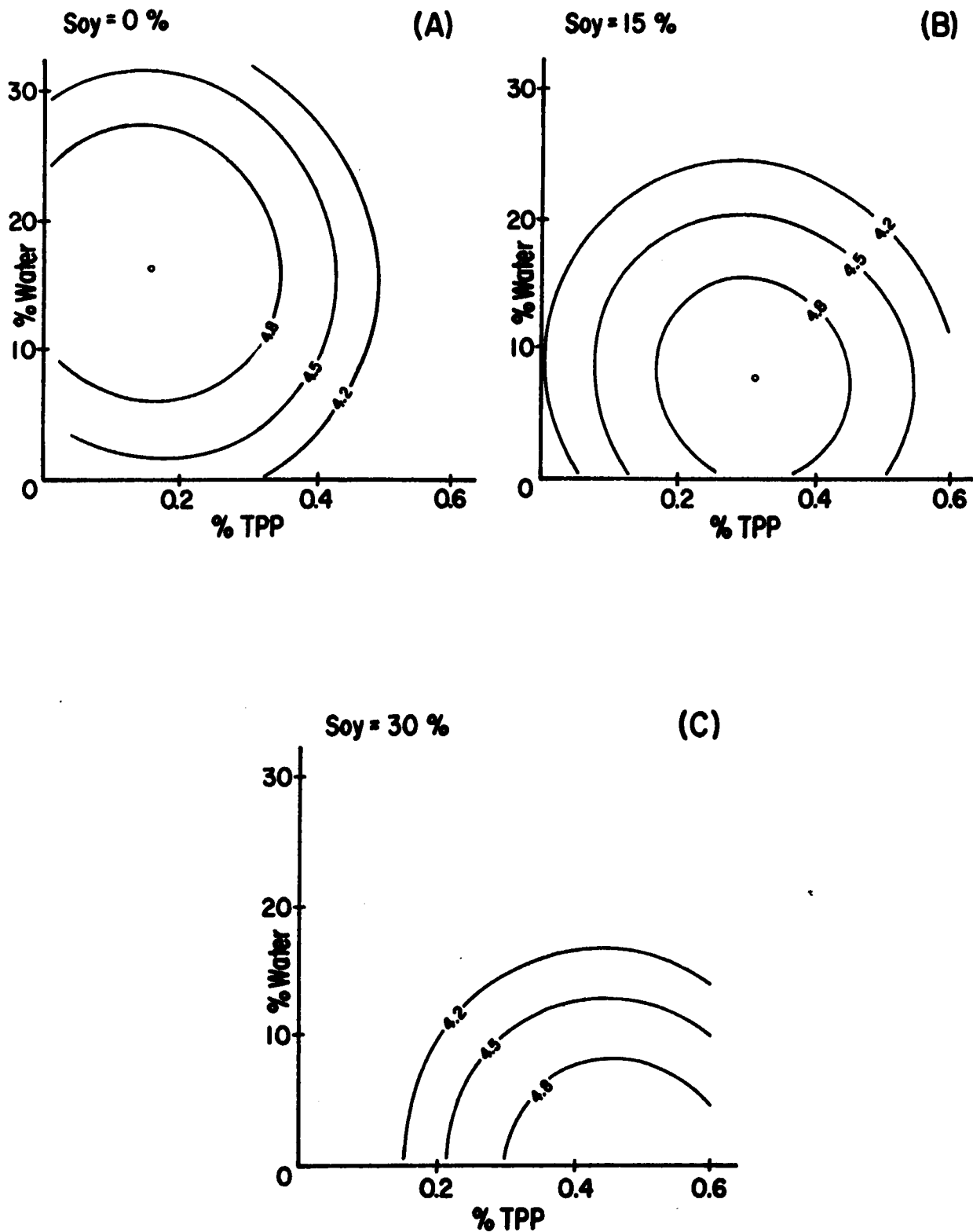
Figure 1. Response surface contours for texture panel scores at 0% soy (A), 15% soy (B), and 30% soy (C).
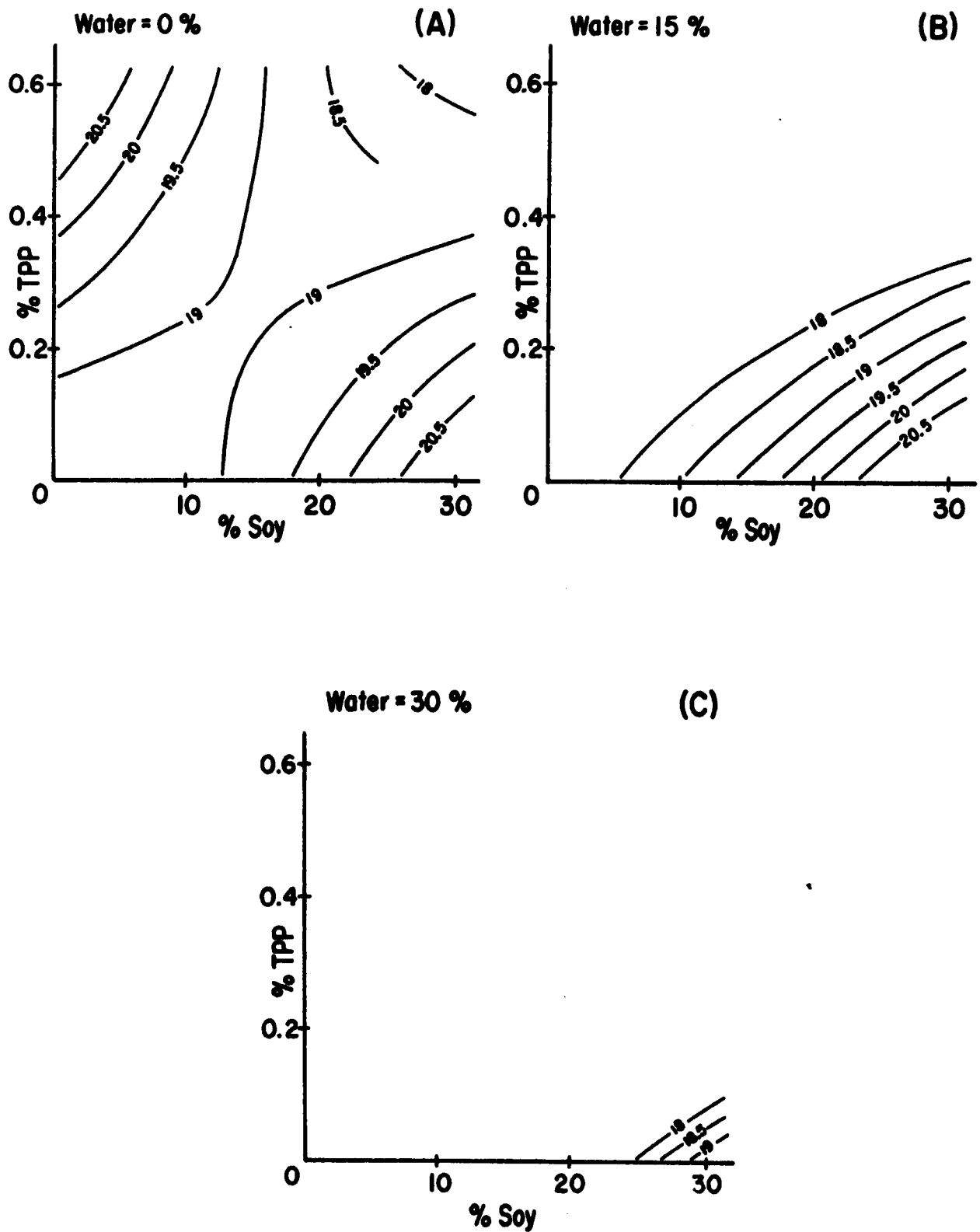
Figure 2.   Response surface contours for shear force values at 0% water
(A), 15% water (B), and 30% water (C).
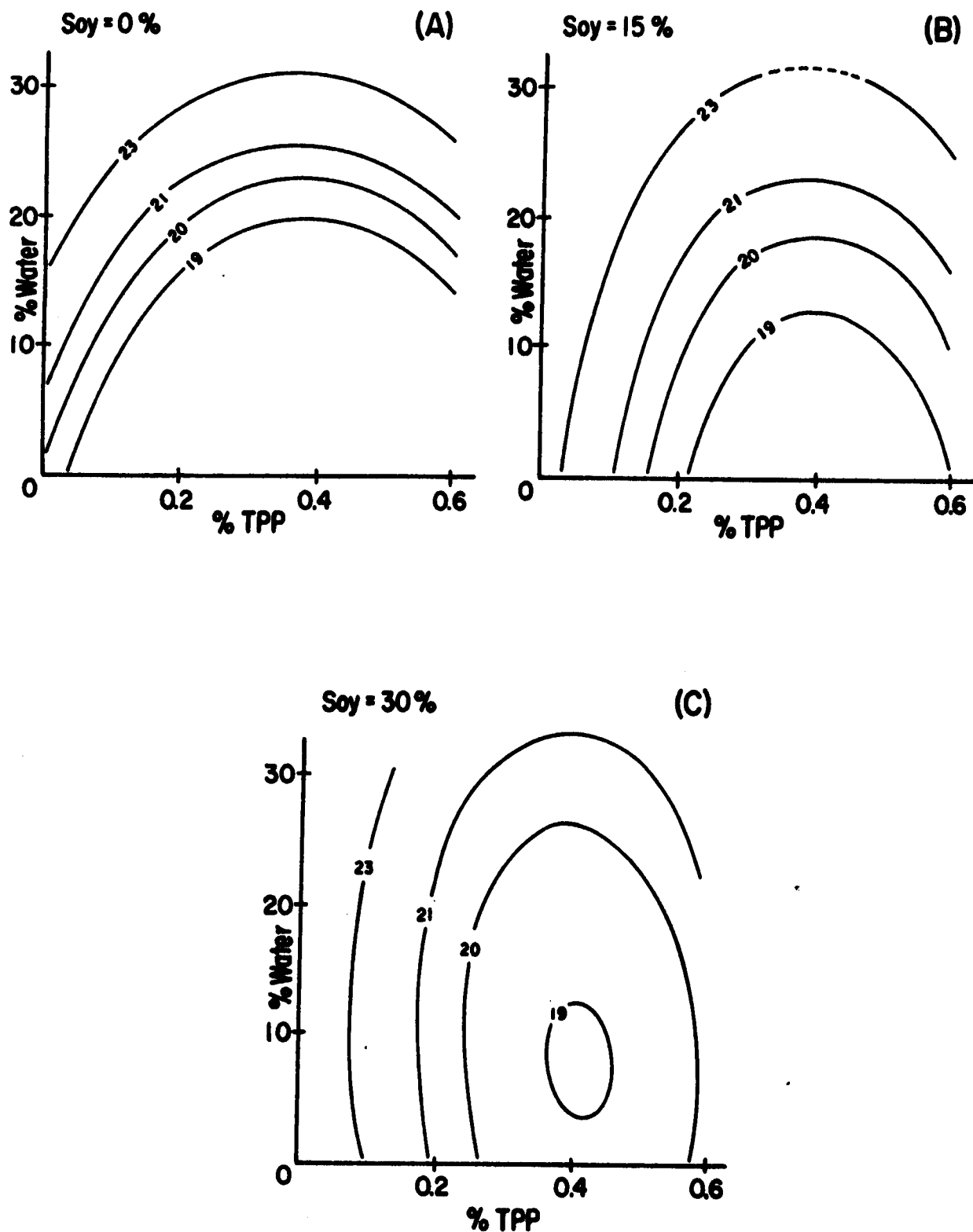
Figure 3.   Response surface contours for cooking losses at 0% soy (A),
            15% soy (B), and 30% soy (C).

STORAGE STABILITY OF A "SEA DOG" MADE FROM MINCED MULLET

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The price of animal protein foods is expected to increase considerably in the future if population growth trends continue. Inflation in the U.S. economy (6) coupled with a growing demand for animal products from other increasingly prosperous countries (20) may boost prices even higher. These developments provide an incentive to create new products from less expensive protein sources.

In our previous study in these same proceedings ("Sea Dog" made with minced mullet and textured soy flour), a sausage-type product (the "Sea Dog") was developed to increase the acceptability and marketability of mullet, an underexploited fish species. Although plentiful in the Gulf Coast area, mullet is underutilized primarily because it is prone to oxidative rancidity (3) which limits long term frozen storage. A complete description of the problems of mullet utilization can be found in our previously mentioned study.

The sausage product was designed to overcome the drawbacks of the mullet species as well as to be acceptable to the U.S. population. No fat is added to the fish sausages, so they contain fewer calories than similar products currently marketed--an important consideration for many overweight Americans. For example, regular beef and pork sausages may legally contain as much as 30% animal fat. An estimated caloric value for "Sea Dogs" (calculated by the ingredient levels) was only 140 calories/100 grams, a much lower value than that for these typical meat sausages. In addition, fish lipids are highly unsaturated, another nutritional advantage. The protein content of mullet sausages should be equal to or greater than that of meat sausages because the customarily added fat is replaced by textured soy protein. "Sea Dogs" should also be less expensive than their meat counterparts, because the ingredients are less expensive.

The purpose of this study was to estimate the shelf life of the product at 35°F to determine the feasibility of marketing. The storage study was designed to compare the effect of (i) hand filleted or mechanically deboned mullet flesh, (ii) a liquid smoke flavoring at levels of 0% or 0.5%, and (iii) a textured soy flour at the 0% or 19.5% level. Deboned flesh was studied because the recent development of mechanical deboning machines for fish can substantially increase flesh yields (2), thus reducing the cost of raw materials. Liquid smoke flavoring was included because it has been reported to inhibit rancidity development and microbial growth while improving the sensory acceptability (10). Soy protein is currently less expensive than flesh proteins (11) and the price differential is expected to widen with time (6).

MATERIALS AND METHODS

Sausage preparation

Two hundred pounds of mullet was purchased from Placida, Florida, in late March and transported on ice to Gainesville. Half of the fish was

filleted, skinned, and washed by hand. The other portion was halved and gutted by hand before mechanically deboning with a Baader 694(Nordischer Maschinenbau) separator with 5 mm diameter holes. Processed flesh was stored at 35°F until used in sausage formulations. Sausages were prepared the day following fish processing as described in our previous study but with different factor levels chosen from some optimum combinations predicted by the response surface analysis. Constant factors used were 100 ppm sodium nitrite, 1.6% sodium chloride, 0.9% sugar, 1.1% spices, 0.25% sodium tripolyphosphate (food grade, FMI), and 9.7% water. No preservatives were added to any samples other than the 100 ppm sodium nitrite (in all the samples) and 0.5% liquid smoke flavoring (Charsol C-6, Red Arrow Co.) in half of the samples. Eight different sausage samples were prepared as shown in Fig. 1. Textured soy flour (Centex 400, caramel, Central Soya Co.) was always rehydrated as 1 soy:3 water.

Objective evaluation

The microbiological assay included a standard plate count, a presumptive coliform count, and a fungi count. Enumerations were performed according to accepted procedures (18) on two duplicates and two replicates for each sample. Twenty-five gram samples were aseptically removed from sausages stored at 35°F and blended with 225 grams of sterile diluent. One mililiter aliquots were plated from serial dilutions using media (Difco Laboratories) prepared according to the manufacturer's instructions. Plate count agar incubated at 35°C for 48 hours was used for the standard plate count, and results were expressed as aerobic plate count per gram at 35°C (APC/g). Yeasts and molds were enumerated using plate count agar with added antibiotics (chloramphenicol and chlortetracycline HCl at a level of 100 mg/liter each) (8). Plates were reported as colony forming units per gram at 25°C (CFU/g). Presumptive coliform counts were obtained with Violet Red Bile agar incubated at 35°C for 24 hours and reported as presumptive coliforms per gram at 35°C (organisms/gram).

Thiobarbituric acid (TBA) number was determined for the samples as an indicator of rancidity. The TBA analysis was performed according to the method of Yu and Sinnhuber (21) except in sample preparation. (Fifty gram samples were blended with two volumes of cold deionized water). Absorption values were read with a Beckman Model 25 Spectrophotometer. Duplicate samples were averaged and reported as mg malonaldehyde per 1000g sample.

Subjective evaluation

A fresh reference sample was always prepared the day prior to evaluation. The sausages were rated by the same nine judges using the same seven point hedonic scale described before in our previous study ('Sea Dog' made with minced mullet and textured soy flour) where 1 = extremely poorer than reference, 4 = same as reference, and 7 = extremely better than reference. Panels were held approximately every seven days.

Statistical design

A 2x2x2 factorial over time was employed to compare the effect of filleted (F) vs. deboned (DB) flesh, soy (S) vs. no soy (NS), and Charsol (C) vs. no Charsol (NC). Linear regressions were calculated by computer using the SAS program package.

RESULTS AND DISCUSSION

Microbiological assays

Bacteria counts were performed on the raw materials as well as the sausage samples. Counts were relatively high (1.0×10^6 APC/g) for the spice mix, a typical result (5). Counts for textured soy were very low (1.3×10^2) probably because of bacterial destruction by the heat employed in the extrusion of such products. Surprisingly, counts for halved mullet (1.3×10^4) were not much higher than for filleted mullet (8.8×10^3). A greater difference was expected because halving requires gutting, which exposes the flesh to the intestinal microbes, while filleting does not. The difference between deboned mullet (1.4×10^5) and ground mullet fillets (1.8×10^4) were also small, in agreement with Martin (12). After cooking at 375°F for 50 minutes, the bacteria counts were extremely low for all sausages with little variations between the treatments on day 1, as shown in Table 1. Bacteria counts showed increasing numbers with storage time and the typical growth pattern of a lag phase followed by a logarithmic increase.

A regression analysis for the bacteria counts showed a highly significant time effect and Charsol effect ($\alpha < 0.01$). Neither the flesh type nor the soy level affected the bacterial counts, confirming the raw materials analysis and in agreement with other researchers (7,17). Therefore, these data were pooled and plotted in Fig. 2 to clarify the Charsol effect. Although Charsol did not initially affect bacterial numbers, a inhibiting effect was apparent with increasing storage time, in agreement with other authors (4,19). Between days 20 and 23, obvious spoilage (odor at approximately 10^7 APC/g and slime formation at approximately 10^8 APC/g) had occurred in non-Charsol samples and so they were no longer analyzed. Similar spoilage occurred in Charsol samples between days 25 and 28. Mullet sausages therefore had a shelf life of approximately 3 1/2 weeks from the viewpoint of bacterial spoilage.

Vacuum packaging has been shown to greatly increase the storage stability of meat products (15) by inhibiting the growth of the more putrefactive organisms, and could possibly be applied to mullet sausages.

Fungi counts were consistently low for four weeks of storage. No growth occurred in any of the samples before the eleventh day, when two samples showed low numbers. After four weeks, average counts were less than 10^3 CFU/g. There were no obvious trends, and statistical analysis was omitted because bacterial numbers caused spoilage long before the fungi could become a problem.

Typical coliforms on Violet Red Bile agar were counted as brick red colonies surrounded by a clear zone of precipitated bile. After four weeks of storage, none of the Charsol samples exhibited typical coliform growth even though they showed obvious spoilage from other microorganisms. The coliform counts became significantly large (10^3 organisms/g) after 40 days of storage. Again, no statistical analysis was performed because coliforms did not present a problem.

TBA analysis

Statistical analysis for the TBA numbers showed a highly significant time effect ($\alpha < 0.01$). Flesh type was significant at the 0.05 level and soy was significant at the 0.01 level. The data was pooled in Fig. 3 to show the effect of the flesh type. Deboned flesh showed a

consistently higher TBA number than filleted flesh, probably because a greater surface area was exposed to oxygen by the deboning process, promoting oxidative rancidity.

Pooled data for the soy effect is shown in Fig. 4. The soy had a slight prooxidant effect, in contrast to results of Kotula et al. (9) and Bowers and Engler (1) for ground beef.

The liquid smoke flavoring (Charsol) had no significant effect on the TBA number. This was unexpected because a preliminary study in November showed greatly reduced TBA values for samples containing 0.6% Charsol. TBA numbers in November ranged from 4-23 over four weeks while those in April ranged from 2-5. This is in agreement with the report of Mendenhall (13) who also found lower TBA values during the spring months. One possible explanation for the discrepancy is the increase in dark flesh (3) and lipid content during the spawning season of November, because the dark flesh contains components capable of catalyzing lipid oxidation. In any case, rancidity was not a problem during April storage and could be controlled in November with 0.5% Charsol. So again the limiting factor was bacterial numbers.

Sensory evaluation

Only Charsol samples were evaluated because preliminary studies had demonstrated the desirable flavor effect of Charsol. Table 2 shows the average sensory scores for the four Charsol samples with their corresponding TBA values and bacterial counts. As expected, flavor panel scores decreased with time, dropping slightly on day 10 and drastically on day 17. This further reduced the sausage shelf-life to approximately two weeks from the flavor viewpoint. Flavor scores showed a good correlation with bacterial growth ($\alpha < 0.05$), and off-flavors were apparent at approximately 4.8×10^5 APC/g. No correlation was found between flavor scores and TBA numbers, in agreement with Moledina et al. (14).

Texture panel scores decreased only slightly with time (Table 2), and no correlations were found for either bacterial growth or TBA values. However, the addition of 19.5% soy significantly improved the texture ($\alpha < 0.01$) over the 0% soy level.

The trends for overall acceptability paralleled the flavor scores, drastically decreasing between day 10 and day 17. Overall acceptability scores also correlated to bacterial numbers (0.01 level) but not to the TBA values, confirming that sensory acceptability was primarily affected by off-flavors resulting from bacterial growth.

CONCLUSIONS

The storage study predicted a two week shelf life for mullet sausages aerobically stored at 35°F. The limiting factor was the development of detectable off-flavors which correlated to total bacterial numbers of 4.8×10^5 APC/g. Fungi and coliforms did not present a storage problem. Rancidity was not apparent in sausages made from mullet in April, and could be controlled in November by the inclusion of 0.5% liquid smoke flavoring. Further research on extension of the product's shelf life is in progress, employing a physical method (vacuum packaging) and a chemical treatment (antioxidant addition).

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Table 1. Effect of storage time (35°F) and treatments on bacteria count (APC/g) in mullet sausage.

Treatments	Day								
	1	4	7	11	14	17	20	23	25
F,NS,NC	5.8×10^2	8.7×10^1	$1.5 \times 10^{4*}$	$9.4 \times 10^{5*}$	1.3×10^6	$3.0 \times 10^{7*}$	1.3×10^7	3.0×10^7	
F,NS,C	6.0×10^2	1.9×10^3	2.1×10^3	3.2×10^3	5.0×10^3	4.5×10^4	1.1×10^7	2.4×10^7	3.7×10^8
F,S,NC	2.3×10^2	5.8×10^2	1.5×10^3	1.1×10^5	5.5×10^5	1.8×10^6	6.5×10^6	2.6×10^7	
F,S,C	4.2×10^2	2.8×10^2	3.6×10^2	6.1×10^3	5.9×10^3	$1.6 \times 10^{6*}$	5.2×10^6	1.2×10^7	1.4×10^8
DB,NS,NC	4.9×10^2	1.0×10^3	1.1×10^3	4.1×10^4	3.9×10^4	8.2×10^4	3.4×10^5	2.6×10^6	
DB,NS,C	3.2×10^2	2.6×10^2	7.7×10^2	2.2×10^4	2.9×10^4	1.6×10^5	1.3×10^6	5.0×10^5	3.4×10^7
DB,S,NC	8.2×10^2	7.8×10^2	9.0×10^2	7.0×10^4	7.2×10^4	$4.8 \times 10^{8*}$	$7.8 \times 10^{8*}$	3.4×10^8	
DB,S,C	5.4×10^2	3.5×10^2	3.7×10^2	7.7×10^3	2.8×10^4	9.7×10^4	3.4×10^5	4.3×10^6	4.6×10^6

*Estimated value.

Table 2. Effect of storage time on average sensory panel scores with corresponding bacterial counts and TBA numbers.

Response	Day 2	Day 10	Day 17
Flavor	4.50	4.07	2.93
Texture	4.58	4.47	4.36
Overall Acceptability	4.53	4.20	3.14
Bacterial Count	3.9×10^2	9.7×10^3	4.8×10^5
TBA Number	2.42	4.68	4.70
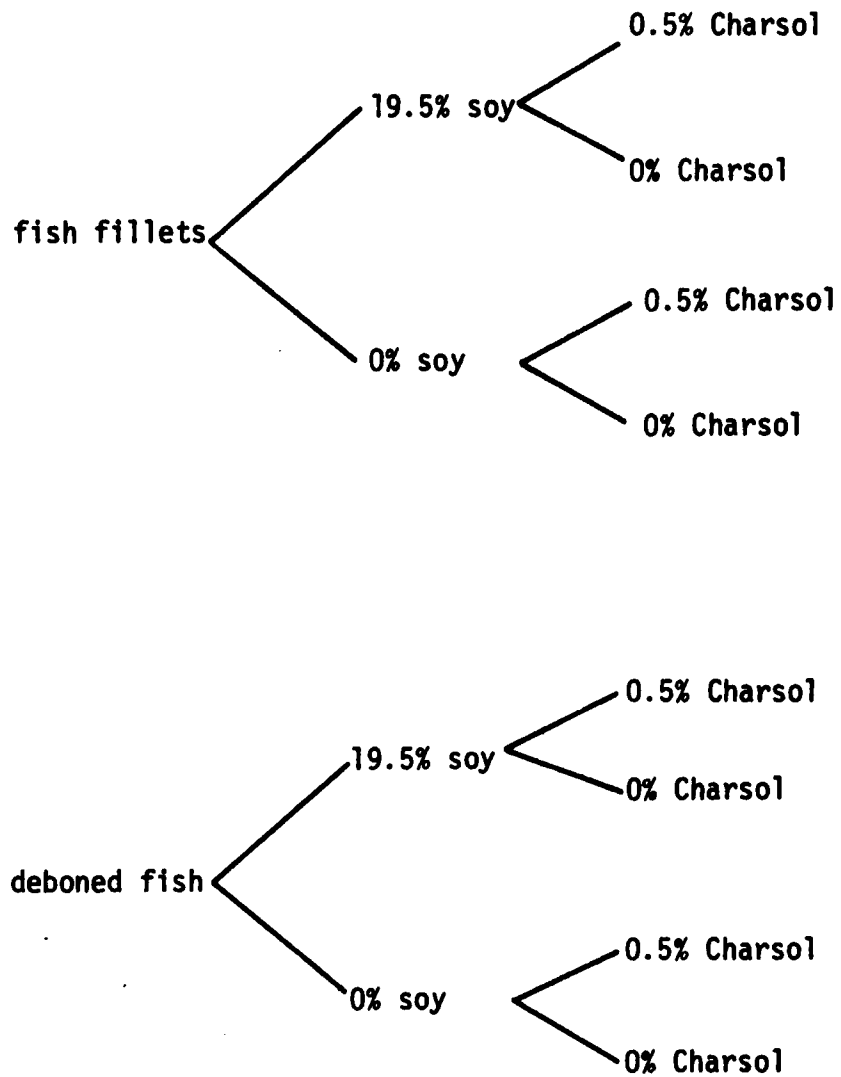
Figure 1. Flow chart for sausage formulations used
in the storage study.
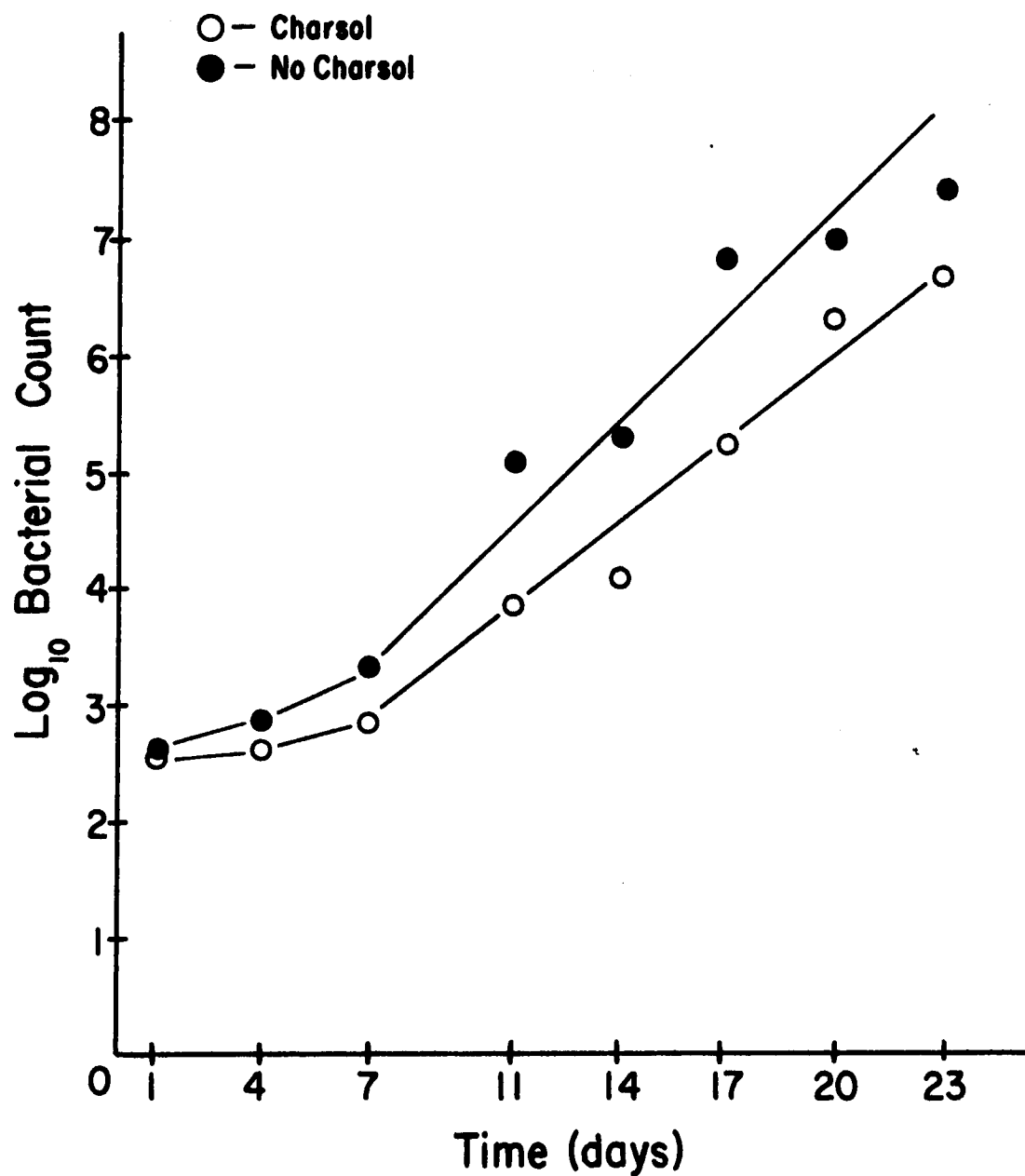
Figure 2. Effect of time and treatment on bacterial growth in sausages with 0 or 0.5% Charsol stored at 35°F. Each point is an average of four samples (16 determinations).
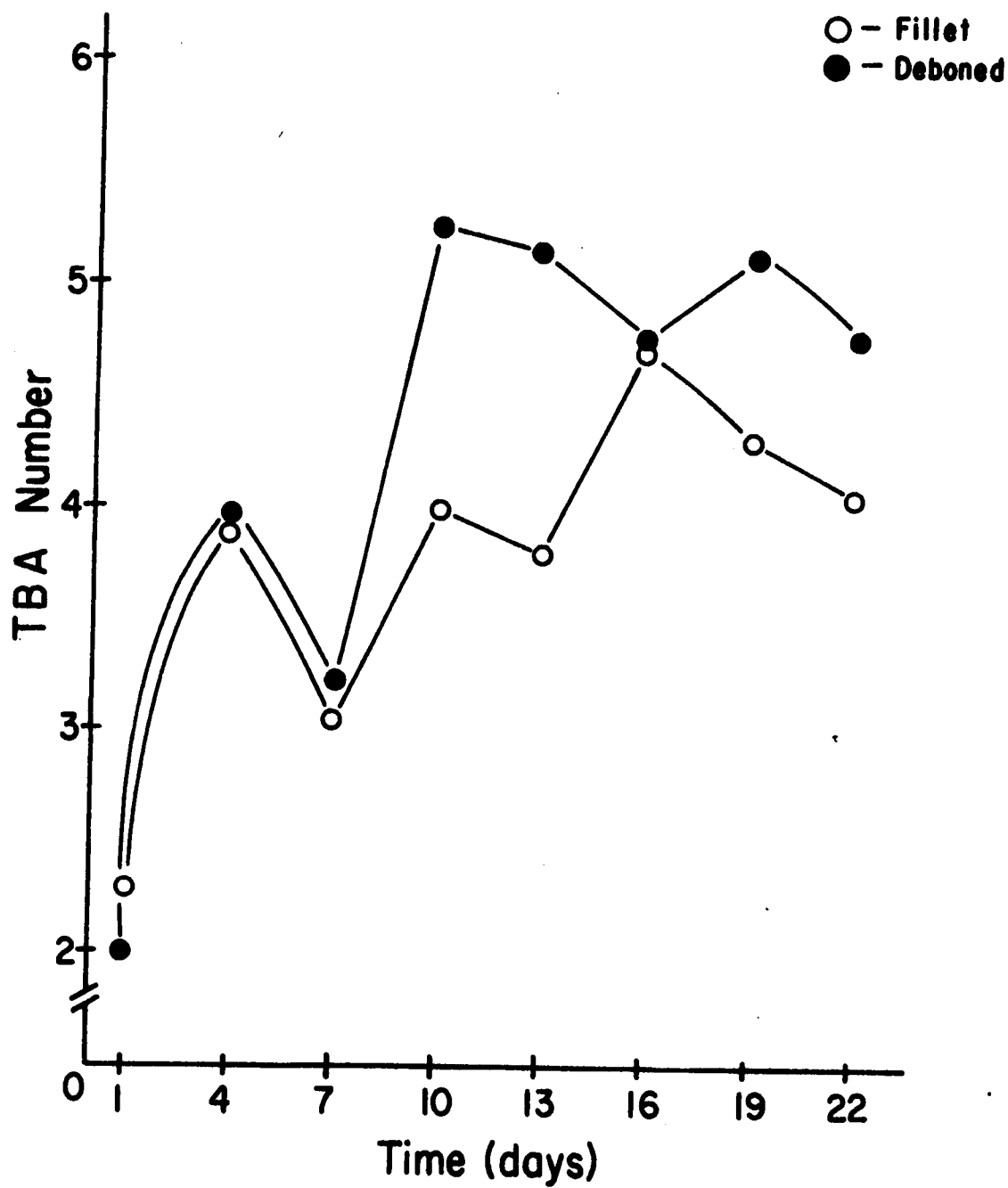
Figure 3. Effect of time and treatment on TBA values for sausages with deboned or filleted fish stored at 35°F. Each point is an average of eight determinations.
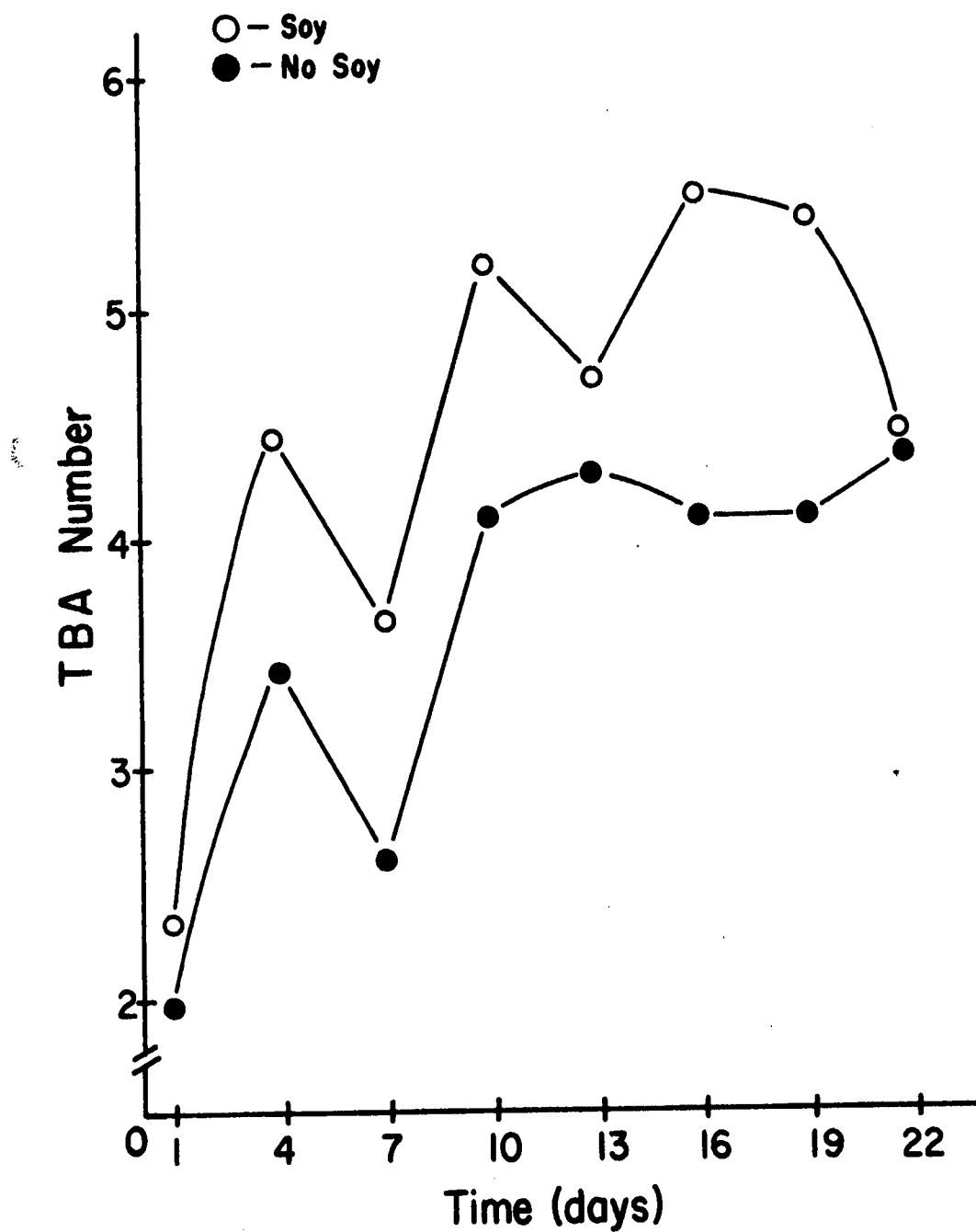
Figure 4. Effect of time and treatment on TBA values for sausages with 0 or 19.5% soy at 35°F. Each point is an average of eight determinations.

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PROCESS DEVELOPMENT FOR A FOREIGN MARKETABLE
FISH PRODUCT FROM UNDERUTILIZED FISH

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The recent expansion of the U. S. fishing jurisdiction to a 200 mile limit is expected to produce a threefold increase in finfish landed from American coastal waters by 1985. Concurrently domestic consumption of finfish is predicted to increase by only one-third (7). Efforts are needed to expand existing markets and to create new markets for popular food fish abundant underutilized species, and so called "trash fish" that are landed incidentally to the primary fishing or shrimping operation.

Export of fishery products is one of the most desirable means of expanding the market for American finfish. Development of fishery products traditionally accepted by foreign cultures from underutilized species of American finfish and from "trash fish" will be considered during a continuing Sea Grant project conducted by the University of Georgia Marine Extension Service. Desirable product characteristics include simplicity of production, stability without costly refrigeration, low transportation costs and high market demand and prices. Some seafood products meeting these requirements included dried fish (with or without salting), dried shark fin, dried squid, and dried seasoned fish flakes (Yu-sone). Existing and future markets for these and similar products can be found in many developing nations, particularly those of Asia and Africa, and among some ethnic groups in the U. S. Groups of general consumers in the U. S. offer possible future markets for several dried products and their derivatives.

Yu-sone, a seasoned semi-dried, and ground fish flake produced for centuries by hand operations in Oriental cultures can be manufactured from fish of any size that have a relatively low fat content. The traditional raw material is bass that is headed, gutted, steamed or boiled, drained, deboned, and disintergrated into small pieces. The loose fish fiber is then sautéed with flour, sugar, soy sauce, and traditional spices until dry and spongy (4). The initial goals of the project were: the

successful mechanization of Yu-sone production, the development of effective quality control systems, the formulation of an optimum mixture of ingredients to consistently produce quality Yu-sone of predetermined moisture content, and to produce a product that is resistant to microbiological spoilage and rancidity development during storage at room temperature. Two species of fish, mullet and croaker, that are landed in abundance along the Southeastern and Gulf coasts of the United States were chosen as raw materials for the pilot studies in Yu-sone production. Both species are underutilized. Croaker (2.2% fat) is representative of fish with relatively low fat values while mullet (16.6% fat) is relatively high in total fat. Two extremes in fat content were chosen to ascertain any differences in final product rancidity during storage at room temperature.

MATERIALS AND METHODS

Materials

Croaker (weighing approximately 250 g) and mullet (approximately 750 g) used in this study originated in Virginia and Florida waters, respectively. Both species were purchased in the freshest state obtainable from a seafood market in Brunswick, Georgia during November and December, 1977. The commercial Yu-sone canned product made in Taiwan was purchased from an Oriental grocery store in New York City. Food grade calcium disodium EDTA was produced by the Dow Chemical Co. (Midland, Michigan). Food grade ascorbic acid was from the Humco Lab. (Texarkana, Texas).

Yu-sone processing

Several important steps in the traditional method of Yu-sone production were modified or replaced to facilitate industrial production, permit the utilization of small "trash fish", create stable and reproducible drying conditions, and allow product monitoring throughout the sauteing operation. Mechanical deboning of dressed fish (using a Miny Fish separator, Yanagiya Machinery Works, Ltd., Ube City, Japan), followed by boiling of the minced flesh, was substituted for the traditional method of boiling dressed fish with subsquent manual separation of bones. To assure stable sauteing conditions a thermostatically controlled (Partlow 30-34 gas control valve and thermostat) gas stove (Prizer Painter Candy Stove B-20) that maintained a wok temperature of 110 + 10 C and a product temperature of 65 + 5 C was installed. Product and wok temperatures were monitored by thermocouples (Omega Copper-Constan with cold junction) attached to a two-pen Omniscribe recorder (Model A5211-1) calibrated to read temperatures ranging from 40 to 200 C. Prior to sautéing the moisture content of boiled and drained minced fish was rapidly determined using an Ohaus Moisture Determination Balance (Model 6010). Moisture content and starting product weight were used to calculate the amount of

additional ingredients to be added during sautéing and the final product weight at a predetermined moisture content. The sautéing operation consisted of manual stirring and loosening of the fish muscle fibers in a wok (50.5 cm in diameter and 12.5 cm deep) supported on the thermostatically regulated gas stove. Sautéing continued at a product temperature of 65 C until the desired final product weight (i.e. calculated final moisture content) was achieved.

Yu-sone production followed the sequence listed below:

(a) Round fish were scaled, headed and gutted.

(b) Dressed fish were mechanically deboned. The recovered minced flesh was collected for boiling or packaged and frozen for later use.

(c) Minced fish from the deboner or previously frozen flesh was boiled in water for forty minutes.

(d) Boiled fish was strained and allowed to drain.

(e) The moisture content of the drained fish was determined using the Ohaus balance.

(f) Drained flesh was placed in a tared wok and weighed.

(g) The wok was slowly heated for approximately four minutes while the minced fish was stirred and separated (Figure 1).

(h) At a product temperature of 45 C the following ingredients were added: 17.5% flour, 10% sugar, 10% salt, and 0.4% ginger (based on the moisture-free weight of the starting material determined in Step f) with continual mixing. When the effect of antioxidants was studied, two batches of mullet (#1 and #4) were prepared by adding the same ingredients as mentioned except salt was reduced to 7.5%, and 0.06% ascorbic acid and 0.05% Na_2EDTA were added in this step.

(i) Fourteen minutes into sautéing, the product temperature was increased to 65 C. Continual stirring and mixing was maintained and the wok was weighed periodically.

(j) After 55 to 60 minutes, the final predetermined product weight was reached (calculated to achieve the desired final moisture content) and sautéing was completed.

(k) The Yu-sone was packaged in aerobic sterile eight ounce plastic containers for later storage studies.

Chemical characteristics

The proximate composition (% moisture, % protein, % fat, and % ash) of all Yu-sone samples and boiled minced flesh samples were determined at zero time (5). Microbiological stability and relative rancidity were determined by total plate counts (2) and by the thiobarbituric acid determination (TBA) (7) respectively at zero time and at three week intervals during three months of room temperature storage. Water activities were determined manometrically on equilibrated Yu-sone samples (1).

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RESULTS AND DISCUSSION

The results of the initial production and storage trials of Yu-sone produced from mechanically deboned mullet and croaker can be summarized as follows:

A. Product Yield:

The generally high moisture content of fish (around 80%) and the small bony fishes used as raw material in this study combined to produce fairly low product yields based on starting weights of fish in the round. Mullet yields ranged from 14 to 18%. Croaker ranged from 10 to 14%.

B. Frozen Deboned Minced Flesh as a Raw Material:

Yu-sone made from frozen minced croaker exhibited no discernable differences in taste or texture from product produced from non-frozen croaker. As shown in Table 1, there were no substantial differences in proximate composition between product #1, #3 (from non-frozen croaker) and products #2, #4 (from frozen croaker). As shown in Table 2, TBA values, an indicator of rancidity, were significantly less for Yu-sone produced from frozen minced croaker than for Yu-sone produced from non-frozen croaker at zero time ($p < 0.01$), at nine weeks ($p < 0.01$), and at 12 weeks of storage ($p < 0.025$) when comparisons were made within each sampling period. When data throughout the storage test were compared, TBA values for product manufactured from frozen minced croaker were significantly ($p < 0.01$) lower than TBA values obtained from Yu-sone manufactured from non-frozen minced croaker. In general, frozen raw materials resulted in products more stable to the development of rancidity. Fish, particularly underutilized species, caught at different seasons and locations could be readily stored at or transported to the production facilities while frozen, assuring a more continuous and possibly a more economic supply of raw materials.

C. Moisture content of the Finished product:

All Yu-sone samples were divided into two groups, stable (moisture<29%) and unstable (moisture>29%). A moisture content of 29% corresponded to a water activity (a_w) of 0.885 (Figure 2). As shown in Table 3, samples with a_w less than 0.875, which included croaker #1,2, and mullet #1,3,4, showed no microbial growth throughout the 12 weeks storage at room temperature. No significant differences ($p < 0.01$) in total plate counts were detected during the three months of storage for products with moisture contents ranging from 9.23% (a_w 0.655) to 27.37% (a_w 0.875). However, samples with a_w greater than 0.875 indicated increased levels of microbial growth after nine weeks (mullet #5), six weeks (croaker #3), and even within three weeks (croaker #4) as shown in Figure 3 and Table 3. Croaker #4 Yu-sone having a moisture content of 36.6% (a_w 0.925) spoiled quite readily. Croaker #3 with a slightly lower moisture content of 36.4% (a_w 0.920) was

stable for only three weeks. Mullet #5 with 29.2% moisture content (a_w 0.885) was stable for six weeks before increased microbial growth was detected after the ninth week of storage. It appears that a maximum water activity of 0.875 is required to resist microbiological degradation of Yu-sone during aerobic storage at room temperatures. Staphylococcus will grow at water activities above 0.86 which corresponds to moisture content of 25% for Yu-sone (Figure 2). Inhibition of molds requires a_w 0.70, (3) which is equivalent to 12% moisture for Yu-sone products under study. The degree of dryness determines product yield, taste, texture, and microbiological stability. The goal of sautéing is to produce a palatable product that is sufficiently dry to maintain microbiological stability at the highest possible yield. Additional preservation such as refrigeration or anaerobic packaging after pasturization is needed to prolong the shelf life of products with a higher a_w. It remains to be determined, whether gains in yield, taste, nutritional value, or energy savings will justify the cost of the treatment and/or packaging.

Comparison of unstable croaker with stable croaker and unstable mullet with stable mullet revealed no significant ($p < 0.01$) differences in protein content or TBA values on a dry weight basis. The commercial product from Taiwan had a very low moisture level, 2.17% (a_w 0.445), and a protein content of 29% (Figure 2 Table 1) which was considerably less than the mean protein values for mullet and croaker Yu-sone which were 67% and 74%, respectively. The low protein content and the less desirable texture of the commercial sample indicated overdrying.

D. Boiled vs. Sautéed Fish:

On a moisture and ash-free basis the average fat content of round mullet 26.5% (9), was reduced to 17.0% after boiling (mullet #4-1 in Table 4) and about 10% after sautéing (mullet #1 and #4 in Table 1), or 16.2% after boiling (mullet #2-0) and 16.0% after sautéing mullet #3 and #5). The average fat content of croaker in the round, 11.3% (9), was reduced to about 4.8% after boiling and about 3.6% after sautéing (Tables 1 and 4). The removal of fat during the boiling and sautéing operation helped reduce the development of rancidity from the oxidation of unsaturated fats and oils in the final product. Sautéing reduced the average protein content (moisture and ash-free basis) of boiled mullet from 82.7% to 68.5% and boiled croaker from 94.4% to 74.1% (Tables 1 and 4).

E. Oily Fish vs. Lean Fish:

Although boiling and sautéing slightly reduced the total fat content of the product, there was a significant difference ($p < 0.01$) in fat content of Yu-sone produced from mullet (mean 13.8%) and from croaker (mean 3.6%) as shown in Table 1. Significant differences in TBA values ($p < 0.01$) for all croaker and mullet samples were detected throughout the storage tests (Figure 4).

Rancidity in mullet samples could be detected organoleptically when TBA values exceeded approximately 130 (Moisture-free basis). Most mullet samples had exceeded this value by the third week of storage, incidating that antioxidants, vacuum packaging, and/or stronger spices were needed to stabilize products from fatty fish. TBA values for all croaker sampled remained low (TBA<80) throughout the storage study, including all microbiologically unstable croaker samples.

F. Effect of EDTA and Ascorbic Acid on Rancidity:

At zero storage time no significant difference ($p < 0.01$) was shown between mullet samples without antioxidants and those with antioxidants (Table 3 and Figure 4). The TBA values of the untreated samples not only exceeded our organoleptic threshold of rancidity (TBA >130), but were significantly greater than ($p < 0.01$) the TBA values obtained for products containing ascorbic acid and Na$_2$EDTA after three weeks of storage. Mullet samples containing 0.06% ascorbic acid and 0.05% Na$_2$EDTA maintained average values below 130 until the sixth week of storage. The average TBA value for mullet samples #1 and #4 (with antioxidants) increased from 156 to 189 between the sixth and twelfth week of storage. A two way analysis of variance revealed a significant interaction ($p < 0.01$) between products with and without antioxidants and time. This interaction supports the assumption that the products containing antioxidants will eventually reach the same TBA value of untreated samples which stabilized around 220. Further studies utilizing different varieties of antioxidants at several concentration levels are needed to determine if the addition of antioxidants is a practical solution to rancidity development in Yu-sone produced from fish with relatively high fat contents (6).

CONCLUSION

The successful three month room temperature storage of Yu-sone produced from the minced flesh of croaker that had been mechanically deboned has demonstrated the feasibility of mechanized Yu-sone production from at least one underutilized species of American finfish. Freezing of the recovered minced flesh did not reduce the quality of the final product and will make it possible to stockpile raw materials during periods of high availability and low prices. Use of the mechanical deboner and the development of quality controls during sautéing that enable repeated production of a final product of predetermined moisture content requires only the development of a mechanical sautéing device for complete mechanization of the process. The development of such a device is now being investigated by this laboratory.

Fish of low fat content and fine muscle fiber are preferred raw materials for Yu-sone production. Species in addition to croaker that may be well suited to production and are available off the Southeastern coast of the United States include spot, whiting, trigger fish, red porgy, sea trout, grouper and black sea bass. These finfish and several other possible raw materials that include shark, skate, and ray are presently being screened for suitability to Yu-sone production.

Early development of product rancidity becomes a problem when oily fish such as mullet are used as a raw material. The incorporation of vacuum packaging or the use of antioxidants and/or additional spices are possible solutions to product oxidation during storage. Microbiological stability for three months of aerobic storage was achieved at moisture contents below 27% and a_w below 0.875. However, longer storage tests at lower moisture levels are needed, along with anaerobic studies, to determine the feasibility of vacuum packaging for room temperature storage.

Successful commercial production of Yu-sone would increase the demand for underutilized fish species landed in the U. S. while introducing a new industry to coastal areas suited to labor intensive or mechanized production that requires relatively small initial investments. Economic and marketing analyses will be needed to determine the size of the initial investment, the amount of Yu-sone production, and the quantity of round fish available at a given price, required to realize an acceptable profit.

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FIGURE 1. Typical "Yu-Sone" Sautéing Curve.

Legend:
- - - - Wok Temperature
——— Product Temperature

① Boiled Fish Added
② Ingredients Added
③ Product Temperature Increased to 65 C
④ Sautéing Completed

(Temperature axis, °C): 115, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20

(Time axis, MINUTES): 0, 10, 20, 30, 40, 50, 60

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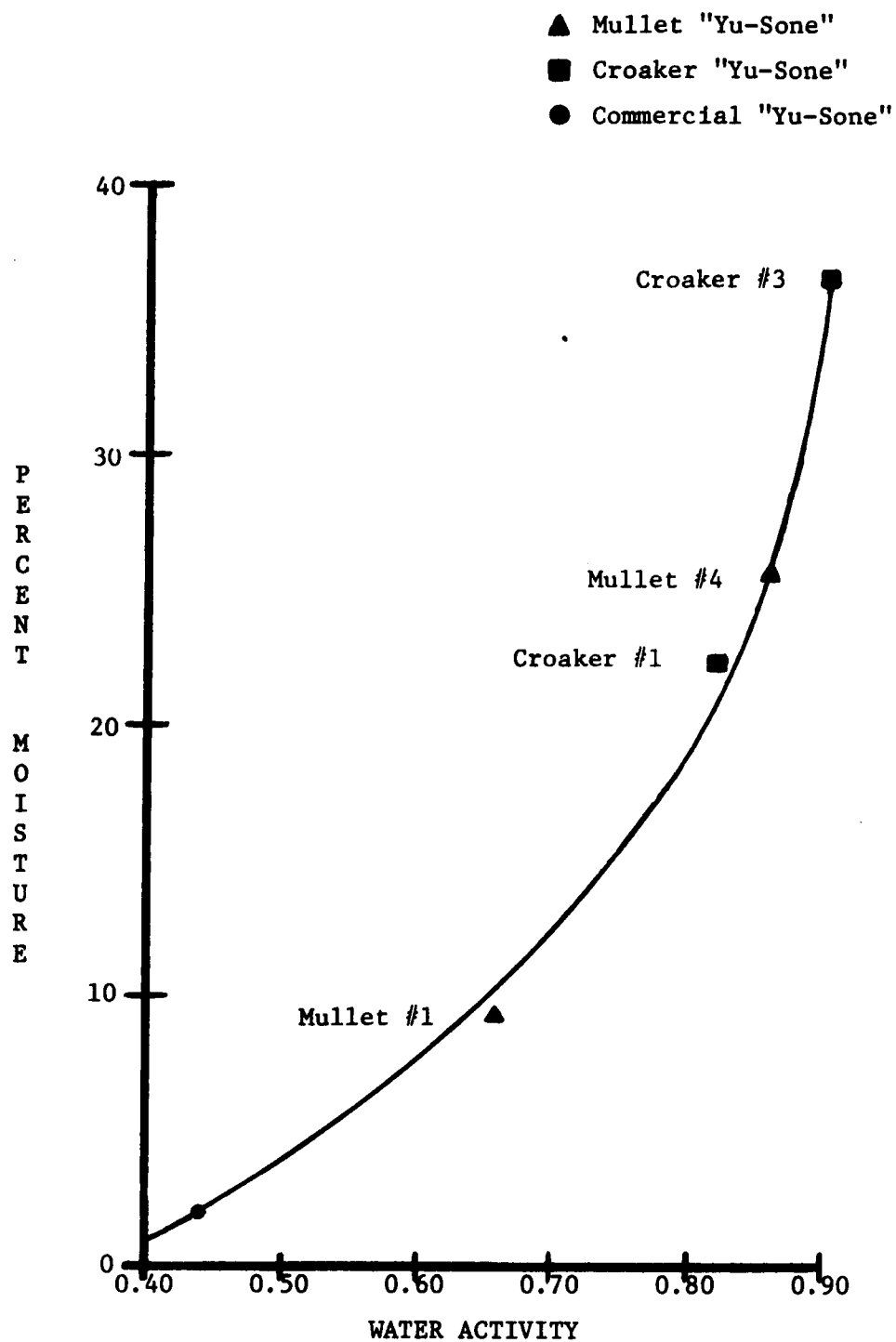
FIGURE 2. Percent Moisture vs. Water Activity in "Yu-Sone".
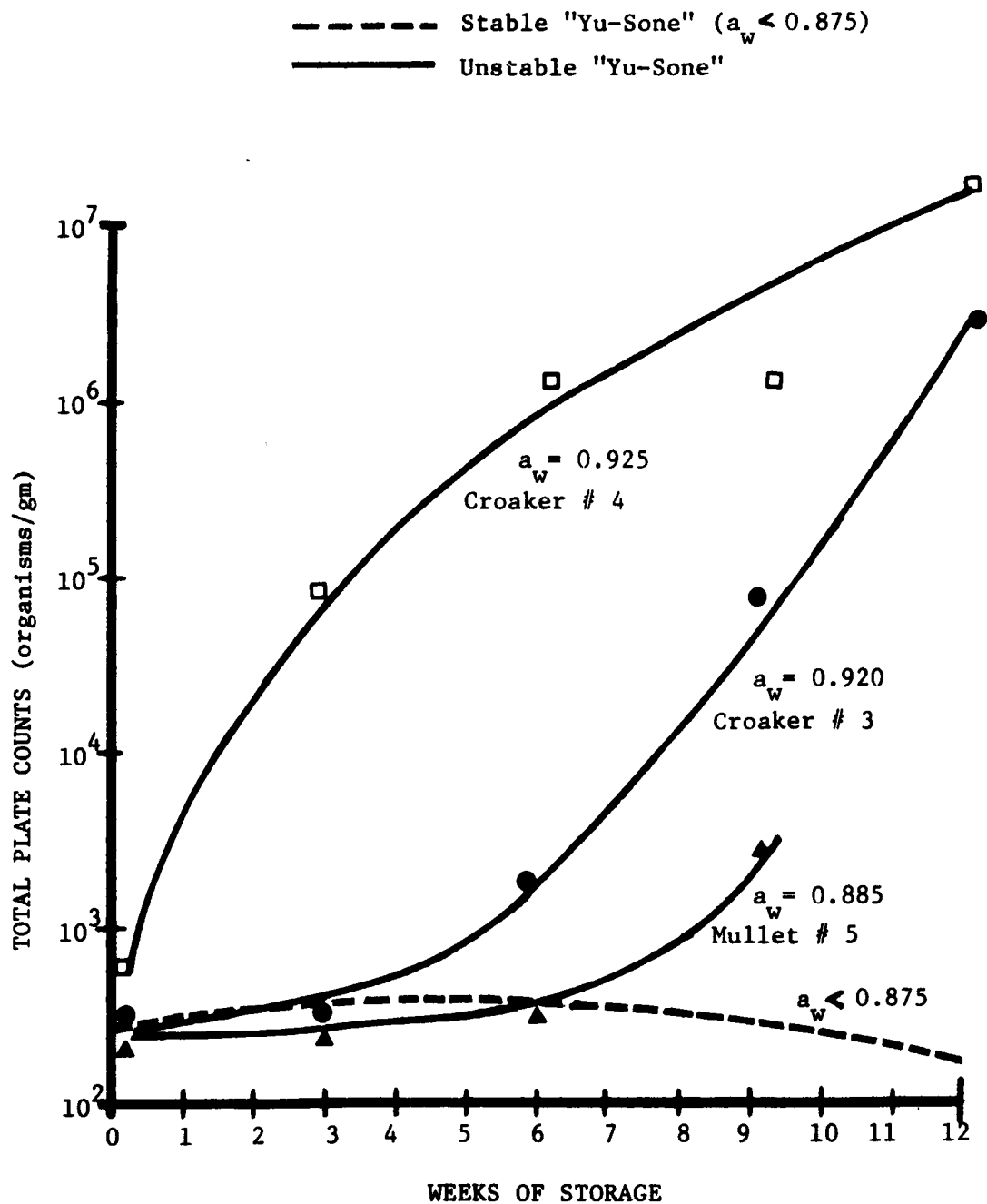
FIGURE 3. Average Total Plate Counts for Stable and Unstable "Yu-Sone" During 12 Weeks Storage.
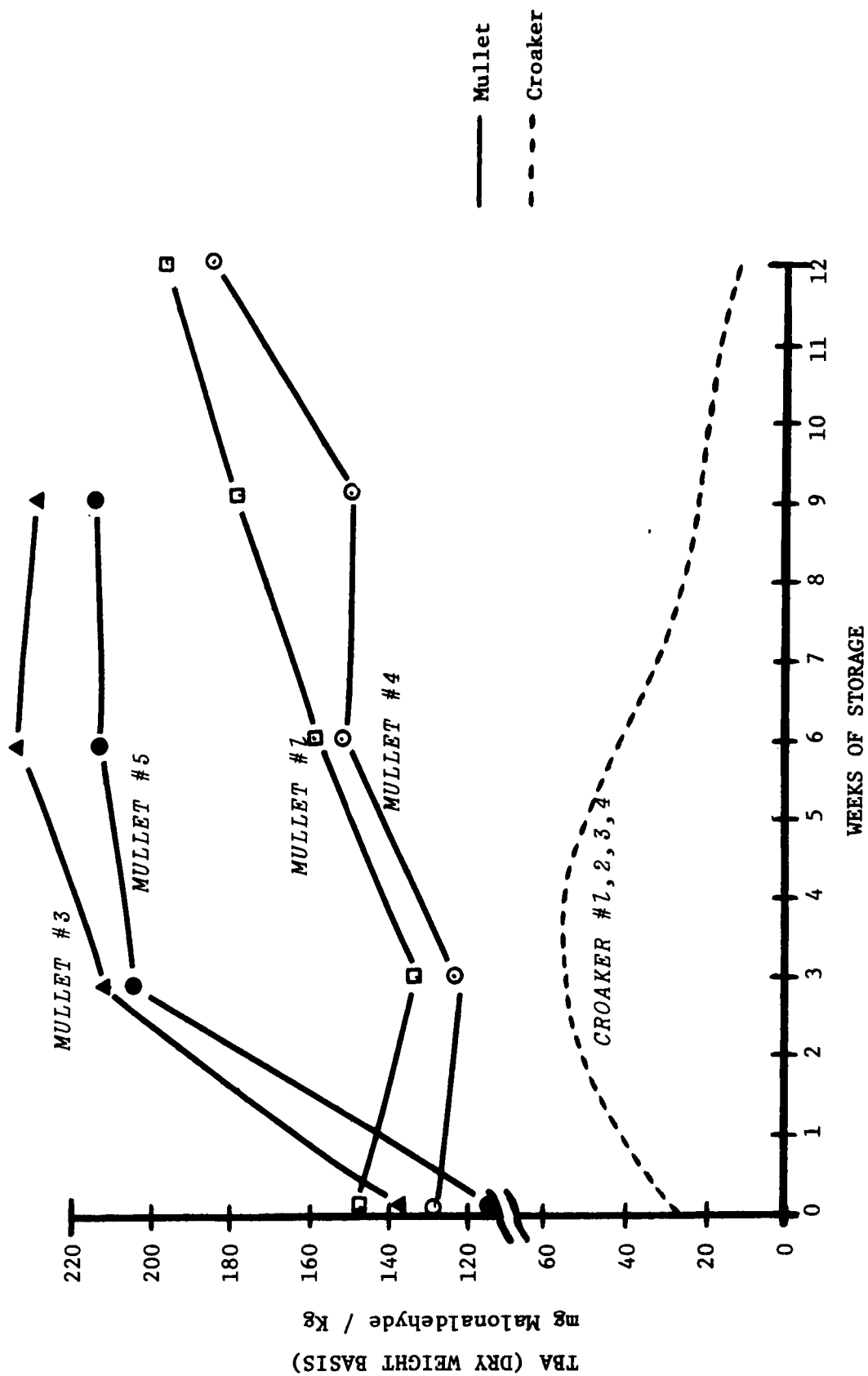
FIGURE 4. Average TBA Values for Mullet and Croaker "Yu-Sone" During 12 Weeks of Storage.

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TABLE 1

"YU-SONE" PROXIMATE COMPOSITION[1]

Parameter	Commercial Taiwan	Croaker[2] #1	Croaker[3] #2	Croaker[2] #3	Croaker[3] #4
% Moisture[4]	2.17	22.09	27.37	36.39	36.64
% Ash[5]	6.77	11.25	10.22	11.20	10.19
% Protein[6]	29.44	73.49	74.45	73.91	74.62
% Fat[6]	31.35	3.39	3.83	3.65	3.59
Parameter	Mullet[2] #1	Mullet[2] #2	Mullet[2] #3	Mullet[2] #4	Mullet[2] #5
% Moisture[4]	9.23	21.01	21.89	25.63	29.15
% Ash[5]	7.27	8.96	8.55	7.32	8.26
% Protein[6]	68.82	74.85	64.77	67.27	66.83
% Fat[6]	10.25	16.04	16.47	9.55	16.60

1. Each data point is the average of two values
2. Sample refrigerated after deboning
3. Sample frozen after deboning
4. Wet basis
5. Moisture free basis
6. Moisture free ashless basis

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TABLE 2

"YU-SONE" TBA VALUES DURING STORAGE[1]

C R O A K E R

Parameter	Unfrozen			Frozen		
	#1	#3	Mean	#2	#4	Mean
a_w	0.830	0.920[2]	----	0.875[2]	0.925[2]	----
Storage Time: (weeks)						
0	34.4	33.8	34.1	8.9	9.0	8.9
3	71.3	46.3	58.7	61.5	40.9	51.2
6	36.9	38.2	37.5	37.3	33.7	35.5
9	27.3	27.3	27.3	21.3	17.0	19.1
12	22.0	23.9	22.9	8.9	16.1	12.5

M U L L E T

Parameter	w/o Antioxidants			w/ Antioxidants		
	#3	#5	Mean	#1	#4	Mean
a_w	0.830[2]	0.885[2]	----	0.655	0.855	----
Storage Time: (weeks)						
0	132.1	116.2	124.2	145.3	129.7	137.5
3	210.3	204.7	207.5	131.9	121.7	126.8
6	232.6	212.5	222.6	159.5	151.9	155.7
9	226.2	212.5	219.4	177.3	144.1	160.7
12	----	----	----	194.6	182.8	188.7

[1]Each data point is the average of two TBA values on a moisture free basis, mg malonaldehyde/kg.

[2]Estimated from Figure 2

TABLE 3

"YU-SONE" TOTAL PLATE COUNTS DURING STORAGE[1]

Parameter	STABLE					UNSTABLE		
	Croaker[2] #1	Croaker[3] #2	Mullet #1	Mullet #3	Mullet #4	Croaker[2] #3	Croaker[3] #4	Mullet #5
a_w	0.830	0.875[5]	0.655	0.830[5]	0.855	0.920	0.925[5]	0.885[5]
Storage Time: (weeks)								
0	405	(220)[4]	300	(295)[4]	310	360	(225)[4]	400
3	320	365	345	(110)[4]	(225)[4]	320	8.20×10^4	(205)[4]
6	535	330	310	(275)[4]	300	1.42×10^4	1.34×10^6	(235)[4]
9	400	(250)[4]	415	(210)[4]	(105)[4]	6.05×10^4	1.44×10^6	2.09×10^3
12	305	(130)[4]	(195)[4]	---	(170)[4]	1.17×10^6	6.51×10^7	---

1. Each data point is the average of two values, organisms/g
2. Sample refrigerated after deboning
3. Sample frozen after deboning
4. Estimate < 30 colonies/plate
5. Estimated from Figure 2

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TABLE 4

PROXIMATE COMPOSITION OF MINCED FLESH AFTER BOILING[1]

Parameter	Croaker[2] #3-1	Croaker[3] #4-2	Mullet #2-0	Mullet #4-1
% Moisture[4]	76.36	77.04	68.80	71.36
% Ash[5]	2.25	2.28	1.23	1.68
% Protein[6]	95.74	93.07	83.03	82.35
% Fat[6]	4.67	4.86	16.23	17.01

1. Each data point is the average of two values
2. Sample refrigerated after deboning
3. Sample frozen after deboning
4. Wet basis
5. Moisture free basis
6. Moisture free ashless basis

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STUDIES ON SHRIMP BY-CATCH
UTILIZATION IN MEXICO

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There is growing interest in the improved utilization of fisheries resources, a high proportion of which is currently wasted (8). In addition to the need to upgrade fish presently used for conversion to animal feed, appropriate technology is required in order to utilize those marine resources which are known but not exploited at all. The situation is of particular concern in developing countries where there is a pressing demand for increased food supply and greater self-sufficiency in food production.

A marine resource which remains largely unutilized is the shrimp by-catch. Appreciable quantities of fish and other marine organisms are caught incidentally in the shrimping operation and this by-catch is generally discarded at sea. It has been estimated that at least 3 to 4 million tons of by-catch is wasted each year (9) and some authorities calculate the annual loss to be as high as 5 or 6 million tons (1, 13). Thus, the shrimp by-catch could make a significant contribution to total world fishery resources.

In view of the lack of information on by-catch utilization and the need to recover the resource for human food, efforts which may contribute to a satisfactory solution have been encouraged (9). Ideally, studies would be undertaken in an appropriate area of a developing country where the demand for the resource is greatest. On this basis, developmental work was initiated in Mexico at Guaymas on the Gulf of California in May 1977. The present paper examines the yields and composition of by-catch recovered during commercial shrimping operations in the Gulf. In addition, it appraises the application of low cost salting techniques (3, 5, 6, 16) to by-catch fish as a convenient means of processing the material into acceptable food products.

MATERIALS AND METHODS

Sampling Procedure
For the collection of samples, voyages were undertaken aboard commercial shrimping vessels, fishing in the Gulf of California, at

approximately monthly intervals between August 1977 and February 1978. The duration of the sampling voyage was normally 3-4 days and the following data was recorded at sea for each trawl: (i) relative weights of shrimp and by-catch, (ii) sampling time and duration of trawl, (iii) location, (iv) depth of trawl and (v) water temperature. About 20-30 kg of by-catch were taken at random from each trawl and stored in sacks, either frozen or iced, for subsequent study in the laboratory.

Evaluation of By-catch Composition and Characteristics

Compositional studies have considered only the fish species since these constitute the bulk of the by-catch and are presently of greater interest for processing. Each organism recovered in the trawl samples was classified according to the keys provided by Jordan and Everman (10), Lindbergh(12) and others (14, 17, 19). The weights and lengths of individual fish in the samples were recorded. Length was measured as the distance between the mouth and the anterior edge of the caudal fin.

Selection of Raw Materials for Processing

Two samples of mixed by-catch, recovered from commercial vessels, were used for processing studies. The samples had the following composition:- Sample A: Pacific sand perch (Diplectrum pacificum) 4.8 kg; sharpnose lizard fish (Synodus scituliceps) 3.8 kg; mojarra (Eucinostomus spp.) 3.2 kg; flounder (Citharichthys sordidus) 2.9 kg; red goatfish (Pseudopeneus grandisquamis) 1.4 kg; Pacific porgy (Calamus brachysomus) 1.4 kg; grunt (Orthopristis cantharinus) 1.0 kg; spotted sand bass (Paralabrax maculatofasciatus) 1.0 kg; sicklefin smoothhound shark (Mustelus spp.) 0.7 kg; Cortez grunt (Lythrulon flaviguttatum) 0.6 kg; Pacific sardine (Sardinops sagax) 0.4 kg; chihuil catfish (Bagre panamensis) 0.3 kg; tiger snake eel (Myrichthys tigrinus) 0.3 kg. Sample B: Salema (Xenistius californiensis) 5.2 kg; mojarra (Eucinostomus spp.) 5.2 kg; Pacific porgy (Calamus brachysomus) 0.7 kg; guitarfish (Rhinobatus productus) 0.5 kg; spotted sand bass (Paralabrax maculatofasciatus) 0.5 kg; grunt (Orthopristis cantharinus) 0.4 kg; gafftopsail pompano (Trachinotus rhodopus) 0.4 kg.

Some of the more common fish species were separated and processed individually. These included flounder (Citharichthys sordidus), mojarra (Eucinostomus spp.) grunt (Orthopristis cantharinus) and corvina (Paralonchurus goodei).

Preparation of salt/fish cakes

After thorough washing and draining, elimination of the bones and skin of the by-catch fish was carried out using a Panli model 19 automatic meat and bone separator. The fish comprising sample B were headed and gutted prior to deboning whilst those of sample A were processed as whole fish. The deboned flesh was divided into 500g batches and intimately mixed with fine salt in a Hobart mixer. Salt concentrations of 10%, 13%, 15%, 17%, 20%, 25% and 30% (as percentage of fish flesh) were compared for their effects on product quality. The salt-fish mix was left to stand for about 30 min and then pressed into cakes manually using a simple hamburger press. About 120g of mix was used for each cake. The pressed cakes were either sun-dried on racks at 25-35°C or mechanically dried in heated air at 37°C.

Preparation of salt/fish flours

Fish were partially cooked by immersion for 5 min in boiling water after which the material was drained and ground in a laboratory mincer. The resultant mince was mixed with 6% salt in a Hobart mixer and dried in thin layers in a similar manner to that described for fish cakes. The dried

material was milled to a fine powder using a pilot-scale hammer mill.

Microbiological Analysis

The microbiological quality of the products was assessed using procedures recommended by the International Committee on Microbiological Specifications for Foods (18).

Chemical Analysis

Crude protein (N x 6.25) was determined by the microkjeldahl method (11). A macrodigest was performed using 1-2g sample, the resultant hydrolysate being diluted to 100 ml. Ten milliliter aliquots of this solution were used for distillation. Crude fat was measured by the Soxhlet method using petroleum ether, ash by ignition in an oven at $500^{o}C$ and NaCl by the ammonium thiocyanate and silver nitrate method (15). Moisture content was determined using an Ohaus moisture determination balance at a power of 2.5 watts and a drying time of 12 min.

Acceptability Testing of Salt/Fish Cakes

The organoleptic acceptability of three different types of salt/fish cake were compared. All the cakes were prepared from deboned fish flesh with the addition of 20% salt using the following raw materials:-
(i) mixed by-catch, sample A
(ii) mixed by-catch, sample B
(iii) flounder (*Citharichthys sordidus*)
Prior to testing, the cakes were desalted by soaking for 30 min in 1l of water, drained, and cooked in a further liter of fresh water for 25 min. Responses of 33 panellists to the three cooked products were measured by hedonic scaling, a score of 9 indicating the most favourable response and a score of 1 the most unfavourable response (2).

Acceptability Testing of Salt/Fish Flours

A sample flour was made from mojarra (*Eucinostomus spp.*), selected from recovered by-catch, and incorporated at 5 and 10% levels in maize tortillas. Acceptability testing of these products was undertaken in a Guaymas community. Consumers were asked to taste "tacos" prepared from ground beans and the supplemented tortillas. After tasting, the weight of leftovers was measured and each of the twenty adult participants was questioned according to the scheme described by Poulter and Disney (16). Ten children with ages ranging from 6-12 years were also tested for their response to the products.

RESULTS AND DISCUSSION

Yields and Composition of Shrimp By-catch

Over the full sampling period, data has been collected from a total of 63 trawls. The mean weight ratio of shrimp : by-catch was 1:7.7 with a range of 1:1.3 to 1:33. This agrees with estimates of by-catch yields obtained by other workers (1, 4, 13), although in the present study the wide range of yields is particularly notable.

To date, 82 fish species have been identified in samples of shrimp by-catch taken from the Gulf of California and these are listed in

chronological order in Table 1. Thus, there appears to be appreciable variability in the composition of the material. However, although many different species have been classified, there are a limited number which appear frequently in large quantities. This is indicated by the data of Table 2 which lists the relative proportions of the more abundant fish species in samples of by-catch recovered from four voyages. It may be seen that these eight species comprised 74% of the bulk of the by-catch. The regular predominance of these fish suggests that some sonsistency in by-catchcomposition may be attainable. Table 2 also shows the similarity in the lengths and weights of the fish. The size distribution of all fish present in samples taken from four voyages (53 trawls) is illustrated in Fig. 1. It is clear that the vast majority of the fish (87% of the total) measure between 7cm and 17cm in length. The mean overall length was 12.6cm and the limited number of larger species encountered consisted mainly of sharks, rays, moray eels and guitarfish. The fact that the bulk of the by-catch fish seem to fall within a fairly narrow size range should facilitate some initial sorting of the material. Furthermore, pelagic species were in evidence only occasionally so that the percentage of lipids in the fish tissues is likely to be low. Both these factors should again contribute to increased consistency in the composition of by-catch and any derived food products.

It should be emphasised that, although several of the fish identified in the by-catch samples are commercial species, their extremely small size would preclude marketing as fresh fish. Observations during the study indicated that less than 10% of the fish recovered in the shrimp by-catch could be considered for normal commercial sale and that these are already selected by the crew. The small size and variability in shape of the fish will inevitably impose considerable problems of handling and evisceration. Indeed, in some cases, complete removal of the viscera may not be feasible at all.

It is disturbing that the bullseye puffer (Sphæroides annulatus), the most toxic of the Tetraodontidae, was detected in samples of shrimp by-catch, albeit in very low quantities. Obviously, such fish would have to be removed, probably manually, before any processing of the material could take place.

General Observations on Processing Techniques and Appearance of Products

Using the Paoli deboner, flesh yields of 68-70% and 60-65% were obtained from whole mixed by-catch comprising small fish and larger fish (sharks, rays, guitarfish) respectively. In addition to the high yields, the separated flesh appeared totally free of any bone material.

Observations on the general appearance of resultant salt/fish cakes with different salt concentrations agree in the main with those reported previously for other fish species (16). Salt concentrations of 10% and 13% promoted excessive shrinkage, a gelatinous appearance and a hard texture in the dried cakes. Dried products with 10% salt were also extremely dark in colour. A similar texture was observed, although to a lesser extent, in fish cakes containing 15% salt. Cakes with 20% salt exhibited minimal shrinkage and it appeared that this was the salt concentration required to cause the protein denaturation and loss of water-holding capacity conducive to optimum cake formation. From preliminary tasting trials on the cooked products it was apparent that cakes which had contained 20% salt were palatable irrespective of the raw

material. It was also notable that the salt was efficiently removed during soaking and most of the samples were fairly bland in flavour.

Salt/fish flours prepared from partially cooked whole fish were light in colour with pleasant odour and flavour. Indeed, flours fabricated in a similar manner from deboned flesh were of inferior quality – dark in colour with stronger odour and flavour. Thus, the cooking process would seem to enhance the quality of the final product in this case. This is probably due to the combined effects of partial elimination of enzymatic and bacterial activity and modification of the colour and flavour components of the fish.

Microbiological Quality and Chemical Composition of Products Prepared from Shrimp By-catch.

Total viable counts (TVC's) for fish cakes of various types, stored for periods of 1-4 months at ambient temperatures, are shown in Table 3. It may be seen that all counts were low, the mean overall count being 6.0×10^2 organisms/g. There was no significant difference between the TVC's in fish cakes of different salt concentration above 10% of the fish flesh. Similarly, although higher counts were obtained in one batch of samples from flounder (<u>Citharichthys sordidus</u>), there was no significant difference between the mean TVC's of products made from different fish species.

The mean values of TVC for cakes prepared from whole fish and eviscerated fish were 9.3×10^2 organisms/g (18 samples) and 3.1×10^2 organisms/g (16 samples) respectively. Although both means are relatively low, the value for cakes including viscera was found to be significantly higher ($p < 0.02$). However, the presence of visceral material in fish cakes made from by-catch appears to promote only a very minor increase in TVC in the final product.

Similar data for a variety of salt/fish flours are presented in Table 4. A wider variation in TVC was encountered with counts ranging from 7.0×10^1 organisms/g to 2.9×10^4 organisms/g. Again, however, the total numbers of bacteria were particularly low.

Analysis for the presence of pathogens has indicated complete absence of coliforms, salmonella and shigella in the products tested. Thus, the present analyses agree with the findings of Del Valle et al (7) and Poulter and Disney (16). In particular, they confirm the good microbiological quality of salt/fish cakes made from mixed by-catch and a number of individual fish species, even with the inclusion of viscera.

The proximate composition of the salt/fish cakes and flours is given in Table 5. These products were fabricated from a wide variety of fish species including mixed by-catch samples A and B. The most notable feature was that the results did not vary appreciably, despite the incorporation of different fish into the products. In the main, differences in protein and moisture contents of salt/fish cakes were due to relative variations in their salt content. Thus, although the species composition of the shrimp by-catch is highly variable, the chemical composition of the resultant products appears to be remarkably consistent. Protein contents were predictably high but fairly low fat values were recorded. The latter reflects the predominance of demersal fish in the by-catch, previously mentioned, these having low contents of fat in the muscle tissue. This factor could be advantageous since the problem of rancidity is unlikely to arise during storage of the by-catch products.

Relatively higher protein contents were observed in the flours due to the inclusion of reduced amounts of salt. Since the flours were prepared from whole fish, the presence of bone calcium and phosphorus would account for the high ash values.

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Hedonic Scaling of Salt/Fish Cakes

Table 6 shows the mean scores and standard deviations obtained for the colour, odour, flavour and texture of the three types of cake tested. All mean scores were between 4.2 and 5.8 which indicates a generally indifferent reaction to the products. There were no significant differences between any of the mean scores for different samples. Wide variations in the responses of different panellists to the same sample were also noted. Several panellists described the texture of the cakes as dry and granular, lacking juiciness, and there appeared to be some preference for the flavour of cakes prepared from flounder. Nevertheless, taking into account the form in which the salt/fish cakes were presented to the panellists, the results of this test seem promising. The cakes were by no means totally rejected even though the individuals cooperating in the study were unfamiliar with such a product. Improvements in flavour may be gained by the inclusion of spices or, perhaps, smoking.

It was interesting to note that no significant difference existed between the mean scores for cakes made from whole fish and those prepared from eviscerated fish. Thus, the presence of viscera did not effectively lower the acceptability of the cakes studied in this test. Further trials are being designed to assess the acceptability of the salt/fish cakes when included in locally popular recipes.

Organoleptic Acceptability of Salt/Fish Flours

The results of the acceptability trial on tortillas supplemented with salt/fish flour are shown in Table 7. Clearly, there was wide acceptability of the enriched meal amongst the panellists tested. However, there was some preference for the product with the lower concentration of fish flour (5%). Weighing of the leftovers indicated 43% and 56% rejection of tortillas containing 5% and 10% respectively of fish flour. This seems satisfactory considering that the testing was carried out during mid-morning, which is not the optimum time for consuming "tacos".

A very favourable response to the products was obtained from the children, who were tested nearer mealtime. All expressed appreciable liking for the "tacos", even with 10% fish flour included, and all but one child consumed the whole sample and asked for more. The percentage of leftovers in this case was a mere 6.5%. It would appear, then, that the people tested were not averse to the inclusion of fish flour at low levels in tortillas and in some instances, preferred the new product.

CONCLUSIONS

The mean ratio of shrimp : by-catch obtained in this study was about 1:8. The quantity of shrimp landed annually from the Gulf of California is approximately 20,000 tons indicating a potential by-catch yield of 160,000 tons each year. Although a wide variety of fish have been identified in samples of by-catch, there appears to be a predominance of some eight different species. This, together with the fact that the vast majority of the fish were demersal types, should promote some consistency in the composition of products developed from the material. The relatively small size range of the bulk of the fish may permit some initial sorting but evisceration of fish of the sizes encountered will obviously impose considerable problems.

Fish minces prepared from by-catch by mechanical deboning or partial

cooking and grinding may be fabricated into food products using simple quick-salting techniques. The proximate composition of fish cakes and flours made by these processes was fairly constant despite wide variations in the raw materials selected. This supports the observations from the taxonomic studies mentioned above. The good microbiological quality of the products, even after several months storage at ambient temperature, was confirmed during the study. Moreover, the inclusion of visceral material does not seem to be problematic in this respect.

Preference testing of the salt/fish cakes in the laboratory indicated limited acceptability of the products but further tests are required. In particular, response to incorporation of the cakes in locally popular recipes needs to be assessed. However, trials completed in local communities indicated good acceptability of maize tortillas supplemented with 5% and 10% of salt/fish flour. Development and acceptability testing of such products prepared from by-catch will be the subject of further studies.

The problems associated with the utilization of shrimp by-catch in developing countries involve economics and logistics as well as technology. All these factors demand detailed examination before the resource can be collected as food for human consumption. Nevertheless, the preliminary technological studies reported in this paper demonstrate the feasibility of preparing inexpensive food products from by-catch fish. Studies to encourage recovery of this presently wasted material will thus be continued.

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Table 1   Species of Fish Identified in Samples of Shrimp By-catch
           Collected from the Gulf of California (Aug. 77 - Feb. 78)

Scientific Name	Mexican Common Name	English Common Name
Eucinostomus spp	Mojarra plateada	Pacific flagfin mojarra
Orthopristis cantharinus	Burrito	Grunt
Scorpaena guttata	Lapon	—
Citharichthys sordidus	Lenguado	Flounder
Paralabrax maculatofasciatus	Cabrilla	Spotted Sand Bass
Paralonchurus goodei	Corvina	—
Calamus brachysomus	Mojarron	Pacific porgy
Rhinobatus productos	Pez guitarra	Shovelnose guitarfish
Umbrina xanti	Roncador	Croaker
Bagre panamensis	Bagre	Chihuil catfish
Porichthys margaritatus	Pez sapo	Southern midshipman
Pleuronichthys ritteri	Lenguado	Flounder
Prionotus ruscaris	Lapon	Searobin
Balistes polylepsis	Cochi	Finescale triggerfish
Rhizoprionodon longurio	Cazon	Pacific sharpnose shark
Diplectrum pacificum	Cabaichucho	Pacific sand perch
Synodus scituliceps	Chile	Sharpnose lizardfish
Halichoeres dispilus	Senorita	Chameleon wrasse
Narcine entemedor	Torpedo	Torpedo ray
Chaetodipterus zonatus	Peluquero	Pacific spadefish
Bairdiella icistia	Ronco - Corvineta	Bairdiella
Scomber saponicus	Macarela	Mackerel
Ophichtus zophochir	Morena	Moray eel
Cheilotrema saturnum	Corvina	—
Haemulon scudderii	Mojarra prieta	Grunt
Caulolatilus princeps	Blanquillo	Blanquillo
Trachinotus rhodopus	Pampano	Gafftopsail pompano
Mugil cephalus	Lisa	Striped mullet
Opisthognatos punctatus	Jawfish	Finespotted jawfish
Sphoeroides annulatus	Botete	Bullseye puffer
Urolophus halleri	Raya	Round stingray
Dematistius pectoralis	Pez gallo	Roosterfish
Lutjanus argentiventris	Pargo	Yellow snapper
Euthynnus pelamis	Barrilete	Skipjack
Scorpaena pannosa	Escorpion	Scorpionfish
Fistularia petimba	Pez corneta	Cornetfish
—	Mantaraya	Ray
Umbrina roncador	Roncador	Yellowfin croaker
Pseudupeneus grandisquamis	Chivo	Red goatfish
Albula vulpes	Lisa francesa, Chile	Bonefish
Selene brevoorti	Jorobado	Mexican lookdown
Hoplopargus guntheri	Pargo rayado	Barred pargo
Achirus mazatlanus	Sol	Mazatlan sole
Micropogon megalops	Berrogata	Gulf croaker
Polydactylus opercularis	Raton	Yellow bobo
Peprilus similliums	Palometa	—
Myrichthys tigrinus	Anguila	Tiger snake eel
Cynoscion xanthulus	Corvina aleta amarilla	Orange mouth corvina
Hypsopsetta guttulata	Lenguado	Diamond turbot
Scomberomorus sierra	Sierra	Sierra

Scientific Name	Mexican Common Name	English Common Name
Gnathypops snyderi	Jawfish	Jawfish
Anchoa hellari	Anchoveta	Gulf anchovy
Xenistius californiensis	Pajarillo	Salema
Xystreurys liolepsis	Lenguado	Fantail sole
Paralichthys woolmani	Lenguado	Flounder
Heterodontus francisci	Gato	Horn shark
Otophidium scrippsi	Eel	—
Etropus crossotus	Lenguado	Fringed flounder
Bothus spp.	Lenguado	Flounder
Mustelus spp.	Tiburon mamon	Sicklefin smoothhound shark
Otophidium spp.	Eel	—
Lutjanus guttatus	Pargo flamenco	Spotted rose snapper
Lutjanus peru	Huachinango	Red snapper
Paralabrax auroguttatus	Extranjero	Goldspotted sand bass
Squatina californica	Diablo	Pacific angel shark
Prionotus xenisma	Lapon	Searobin
Orthostoechus maculicauda	Burrito	—
Hippoglossina tetrophthalmus	Lenguado	Fourspot sole
Pomadasys panamensis	Burro	—
Lythrulon flaviguttatum	Burro	Cortez grunt
Polydactylus aproximans	Raton	—
Zapterys exasperata	Pez guitarra, Grande	Guitarfish
Microlepidotus inornatus	Rayadito	Wavyline grunt
Symphurus sechurae	Lengua	Tonguefish
Sphyraena argentea	Picuda	Barracuda
Paralichthys californicus	Lenguado	Flounder
Sardinops sagax	Sardina monterey	Pacific sardine
Urotrygon spp.	Raya	Ray
Urolophus spp.	Raya	Ray
Lophiomus setigerus	Raro	—
Myrophis vafer	Anguila	Estero worm eel
Symphurus fasciolaris	Lengua	Tonguefish

Species	Mean length (cm)	Mean weight (gm)	% of Total
Eucinostomus spp. (mojarra)	12.7	53	26.5
Diplectrum pacificum (perch)	14.0	33	14.0
Citharichthys sordidus (flounder)	12.5	51	12.0
Xenistius californiensis (salema)	13.9	92	5.6
Orthopristis cantharinus (grunt)	13.5	58	4.8
Etropus crossotus (flounder)	7.4	14	3.9
Micropogon megalops (croaker)	8.7	15	3.8
Scorpaena guttata (lapon)	11.0	48	3.5
		Total	74.1

Table 2   Proportions (by number) and mean lengths and weights of fish abundant in samples of shrimp by-catch collected from four voyages (53 trawls).

Raw Material and Treatment				TVC
Fish Species	% Salt	Eviscerated	Cooked	(organisms/gm)
Mixed	20	No	No	1.2×10^2
"	15	Yes	No	4.0×10^2
"	20	Yes	No	3.5×10^2
Cochi (Triggerfish)	20	Yes	No	1.9×10^2
" "	20	No	Yes	1.3×10^2
" "	20	Yes	Yes	2.5×10^2
" "	20	No	Yes	3.7×10^2
Cazon (Shark)	20	No	Yes	2.4×10^2
Lenguado (Flounder)	10	No	Yes	9.6×10^2
" "	13	No	Yes	6.1×10^2
" "	15	No	Yes	4.0×10^3
" "	17	No	Yes	1.4×10^3
" "	20	No	Yes	2.6×10^3
" "	15	Yes	No	8.3×10^2
" "	20	Yes	No	1.1×10^2
Corvina	15	Yes	No	2.9×10^2
"	20	Yes	No	2.5×10^2
"	25	Yes	No	8.0×10^2
Mojarra	15	Yes	No	1.3×10^2
"	20	Yes	No	2.7×10^2
"	10	No	Yes	2.3×10^3
"	15	No	Yes	9.2×10^2
"	20	No	Yes	5.4×10^2
Mixed Sample A	10	No	No	1.8×10^2
" " "	13	No	No	1.1×10^2
" " "	15	No	No	1.2×10^2
" " "	17	No	No	7.4×10^2
" " "	20	No	No	1.4×10^2
" " "	25	No	No	1.4×10^3
Mixed Sample B	10	Yes	No	2.6×10^2
" " "	15	Yes	No	4.0×10^2
" " "	17	Yes	No	1.7×10^2
" " "	20	Yes	No	3.1×10^2
" - " "	30	Yes	No	2.0×10^1

Table 3   Total viable counts (TVC) for salt/fish cakes prepared, using various treatments, from mixed shrimp by-catch and some predominant fish species (after 1-4 months storage).

Fish Species	Storage Time at Ambient Temp.	TVC (organisms/gm)
Mojarra	5 months	2.9×10^4
Lenguado (Flounder)	"	6.1×10^2
Corvina	"	3.8×10^2
Burrito (Grunt)	"	1.0×10^4
Mojarron (Porgy)	"	2.5×10^2
Lapon	"	8.0×10^1
Mixed	"	7.0×10^1
Mixed (raw, eviscerated)	"	1.6×10^3
Lenguado	4 months	5.0×10^2
Corvina (raw, eviscerated)	2½ months	7.1×10^2
Mojarra (raw, eviscerated)	"	1.1×10^2
Lenguado (raw, eviscerated)	"	2.0×10^2
Mojarra	"	2.7×10^2
Mixed Sample A (raw)	2 months	1.4×10^2
Mixed Sample B (raw, eviscerated)	"	1.8×10^2

Table 4   Total viable counts (TVC) for salt/fish flours prepared from mixed shrimp by-catch and some predominant fish species. (With the exceptions indicated, all flours made from cooked and minced whole fish with addition of 6% salt)

Salt/Fish Cakes

	% (^{w}/w)
Moisture (25 samples)	5.0 — 11.9
Crude Protein (25 samples)	40.0 — 56.5
Crude Fat (10 samples)	0.6 — 7.3
Ash (10 samples)	35.6 — 44.0
Salt (10 samples)	27.7 — 39.0

Salt/Fish Flours

	% (^{w}/w)
Moisture (6 samples)	8.2 — 12.4
Crude Protein (6 samples)	53.4 — 64.2
Ash (6 samples)	23.5 — 25.9

Table 5   Proximate composition of salt/fish cakes and flours prepared from shrimp by-catch.

Raw material	Mean Score				Overall Mean Score
	Colour	Odour	Flavour	Texture	
Lenguado	5.8 ± 1.53	5.5 ± 1.78	5.7 ± 1.45	4.7 ± 1.62	5.4
Mixed By-catch A (non-eviscerated)	5.2 ± 1.74	4.2 ± 1.54	4.9 ± 1.58	4.3 ± 1.39	4.6
Mixed By-catch B (eviscerated)	5.5 ± 1.35	4.9 ± 1.60	4.8 ± 1.80	4.8 ± 1.60	5.0

Table 6   Mean organoleptic scores and standard deviations obtained by hedonic scaling of three types of salt/fish cake. (No. of panellists = 33).

Question	Possible Answers	Number of people choosing each answer
(a) Did you enjoy the "tacos"?	Yes No	19 1
(b) Which "taco" did you prefer?	A B No preference	11 8 1
(c) Were the "tacos".... ....than similar "tacos" you have eaten before?	as good not as good better	11 4 5
(d) Would you buy these tortillas if available?	Yes No	19 1

Note: Sample A contained 5% salt/fish flour in tortilla
Sample B contained 10% salt/fish flour in tortilla

Table 7   Results of organoleptic acceptability trials of tortillas supplemented with 5% and 10% salt/fish flour prepared from mojarra (*Eucinostomus spp.*) recovered from shrimp by-catch.
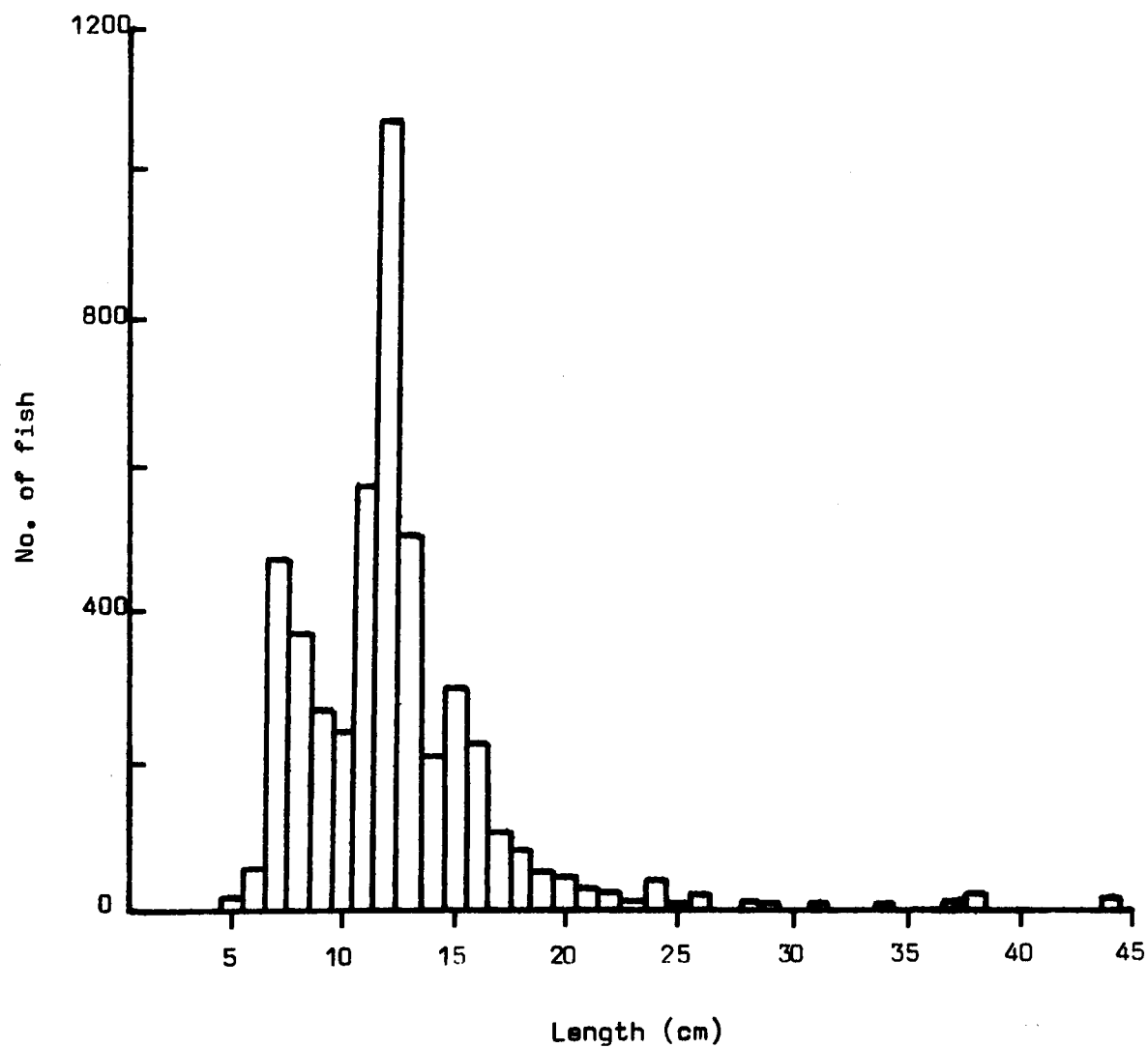
Fig. 1 Size distribution of by-catch fish recovered in samples taken from four voyages (53 trawls)

ABSORPTION OF OXYGEN AS A MEASURE OF FISH MEAL STABILITY

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INTRODUCTION

Oxidation control is essential in producing uniformly high quality fish meals needed by feed manufacturers as an ingredient in efficient rations for poultry and animals. Menhaden is the chief source of domestic fish meal, this fish accounting for 42.3% of U. S. finfish landings in 1977, according to Robert Chapoton of National Marine Fisheries Service.

Wet rendering of menhaden produces condensed fish solubles, fish oil and fish meal, the latter product resulting from the cooking, pressing and drying of the whole fish. "Fish scrap" leaves the dryers at about 10% moisture, is then rapidly cooled and antioxidant applied. Storage is in large piles which may exceed 1,000 tons and which must be constantly monitored to assure acceptably low internal temperatures. Fish scrap is ground into fish meal at time of shipment via truck, railroad car, or barge.

Before 1960 most menhaden processing operations accepted exothermic oxidation of fish scrap as unavoidable and requiring costly multiple handling to avoid overheating during shipment. Serious investigation of antioxidants as a means of reducing oxidation began in the mid-50's.

The Active Oxygen Method for fat stability (11) was used extensively for checking the effect of adding antioxidants to menhaden oil in the belief that the results would provide guidance in stabilizing fish scrap. Figure 1. shows how quickly peroxide values develop in untreated menhaden oil when air is bubbled through it at 97.8o C, as prescribed by the test. What is significant about the antioxidant treated samples shown in Figure 1. is that the moderately effective one (x) turned out to be the one which was selected for treating fish scrap while the other one (y) didn't perform at all under plant conditions. Such failures emphasized the need for conducting laboratory trials on the actual dry systems which constituted the oxidation problem.
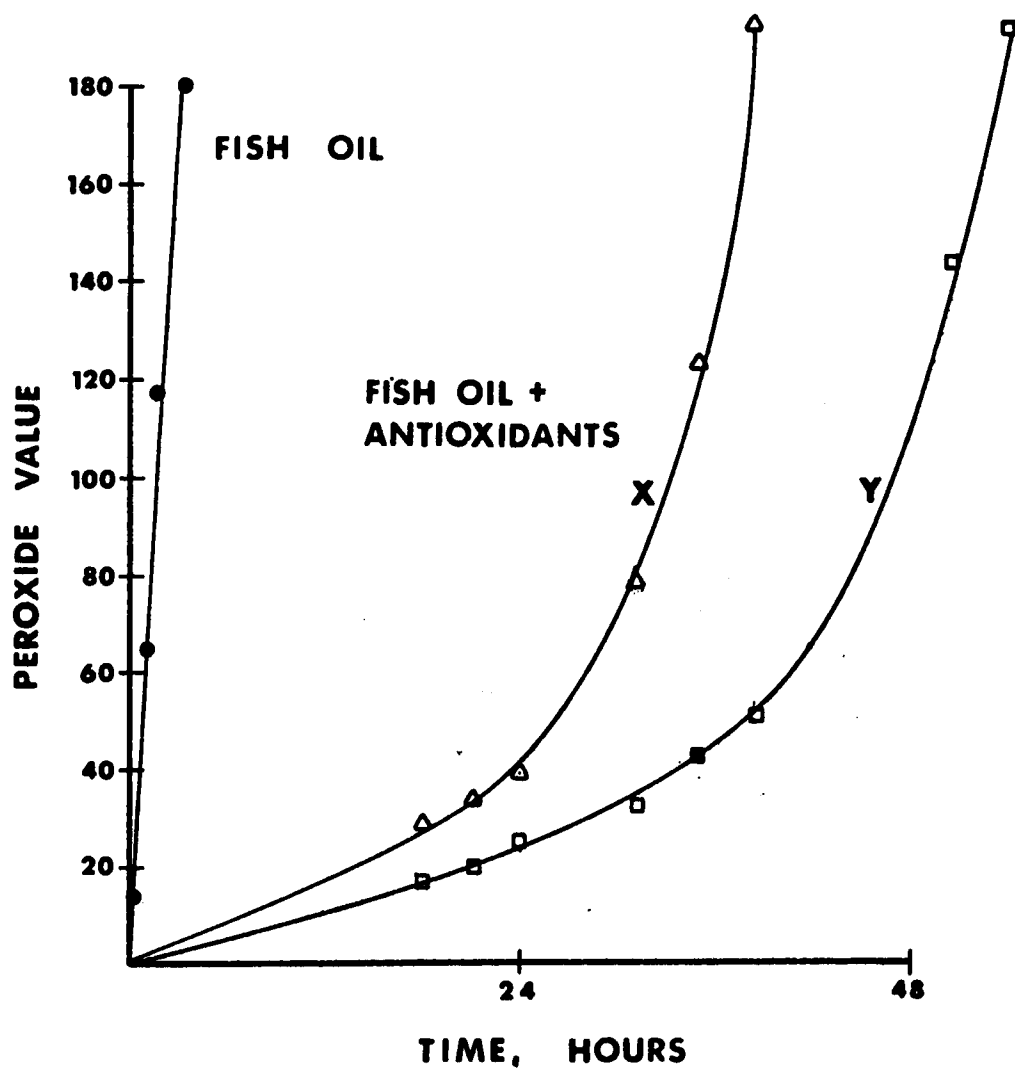
Figure 1. Active Oxygen Method - Menhaden Fish
Oil, with and without Antioxidants.

Marine Chemurgics, Inc.
1957

Another misconception was that the ethyl ether extractable portion of the scrap was responsible for most of the heating problem. Figure 2. shows two typical fish scrap turning sequences as determined in 1957. The scrap having the higher level of free oil did not heat as rapidly as the one with lower free oil content, apparently because the ratio of bound to free lipids was higher in the second instance. This same figure also shows the results of the first successful treatments with butylated hydroxytoluene (BHT).

Gearhart, et al. (5) and others (10) applied a modification of the standard Oxygen Bomb Method for gasolines to the testing of stability of fats and oils, and of foods containing them. Samples weighing 15 to 30 gms. are placed in the glass liner of the bomb which is then sealed and purged with oxygen. It is then brought to standard pressure, inserted in boiling water, and a pressure recorder used to indicate the end of the induction period as indicated by a sudden pressure drop.

The bomb method proved useful in predicting the protective effects of antioxidants applied to fish meal, also providing insight concerning the rapid intake of oxygen by freshly produced fish scrap and the continuing oxidation which can occur if the reactions are not controlled. Figure 3. shows relative pressure drops of freshly produced vs. "cured" fish scrap, both with and without antioxidant additions.

Lea, et al. (7, 8) conducted extensive studies of changes occurring in stored herring meal, reporting that the meals absorbed oxygen rapidly and extensively with levels of 10 to 20 ml. of O_2 (s.t.p.) per gm. of meal being reached the first one or two weeks after manufacture. Adverse effects on lipids and on available lysine which could result from unfavorable storage conditions were brought sharply into focus.

The literature abounds with descriptions of manometric methods employed to measure oxidation and the protective effects of stabilizing systems including discussions appearing in books by Emanuel, et al. (4) and Lundberg (9) and Labuza's (6) discussion of rate of oxidation of an intermediate moisture food with data based on O_2 uptake. Such methods are distinctly related to the Warburg respirometer described by Dixon (3). Blankenship, et al. (2) described the successful use of the Oxygen Bomb Method on peanuts and peanut products while Berner, et al. (1) reported accelerated oxygen uptake by addition of hemin to emulsion systems and measured with a Beckman oxygen analyzer. The validity of the oxygen uptake approach to measuring the effects of antioxidant additions seems sufficiently well established to justify using it for additional work on stabilization of fish meal.

MATERIALS AND METHODS

EQUIPMENT--The apparatus for conducting comparative O_2 uptake determinations consists of a number of self-contained assemblies mounted so that the reaction flasks can be submerged in a constant temperature water bath. Each assembly, as illustrated in Figure 4. consists of a 250 ml. narrow mouth Ehlenmeyer reaction flask, "1"
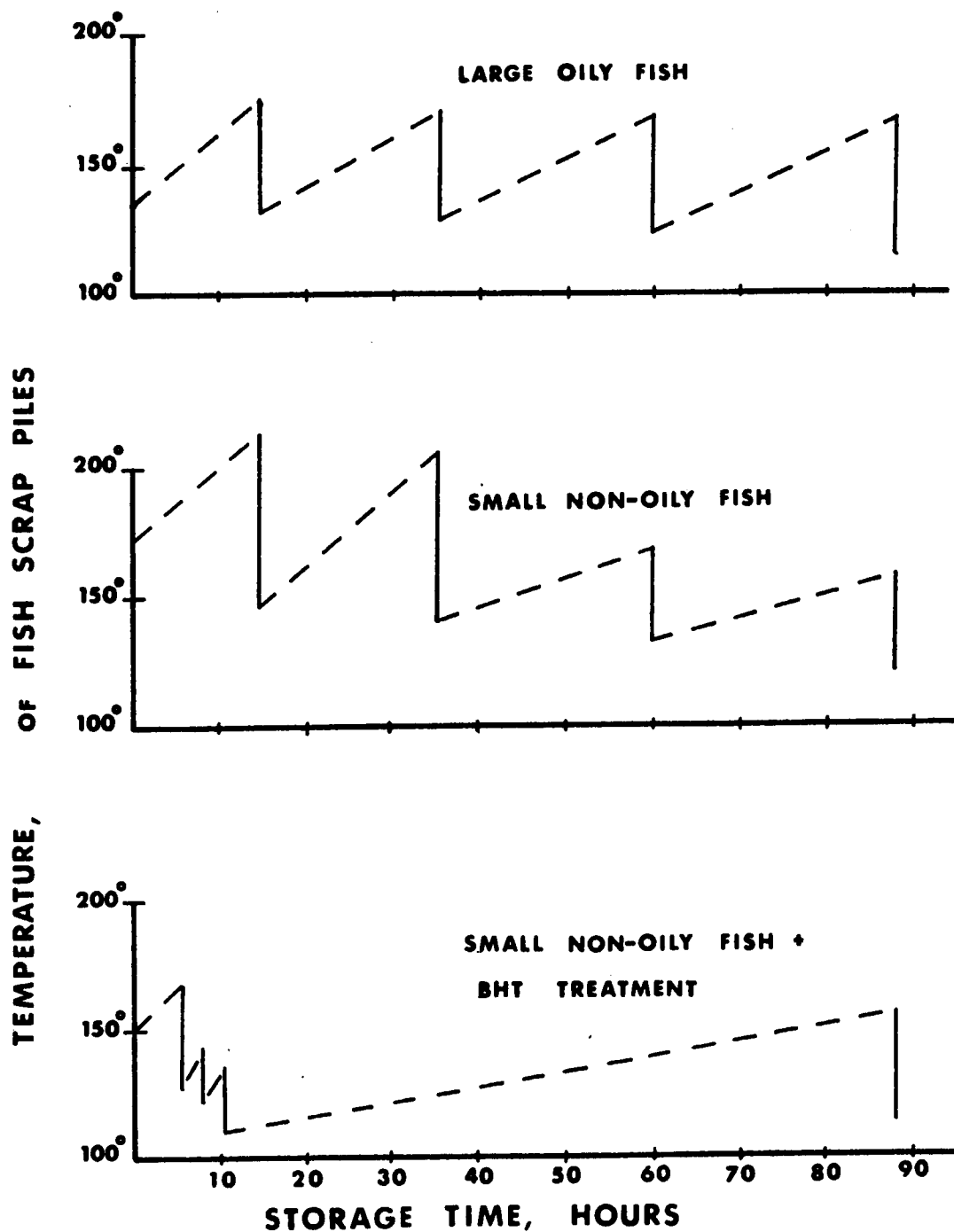
Figure 2. Temperature Increases (°F) in Piles of
Menhaden Scrap and Effect of BHT.

Marine Chemurgics, Inc.
1957

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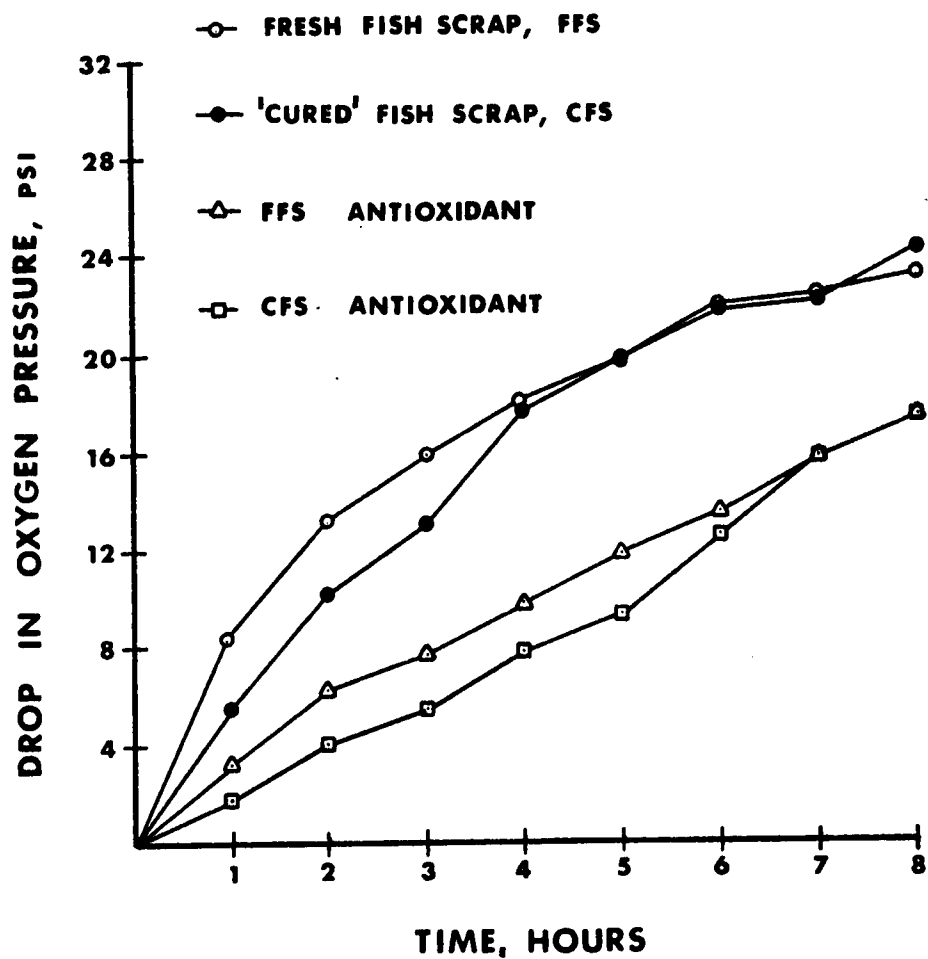
Figure 3. Oxygen Bomb Test Starting at 50 lbs. p.s.i. Fresh vs. Cured Scrap with and without Antioxidant.

Marine Chemurgics, Inc.
1957

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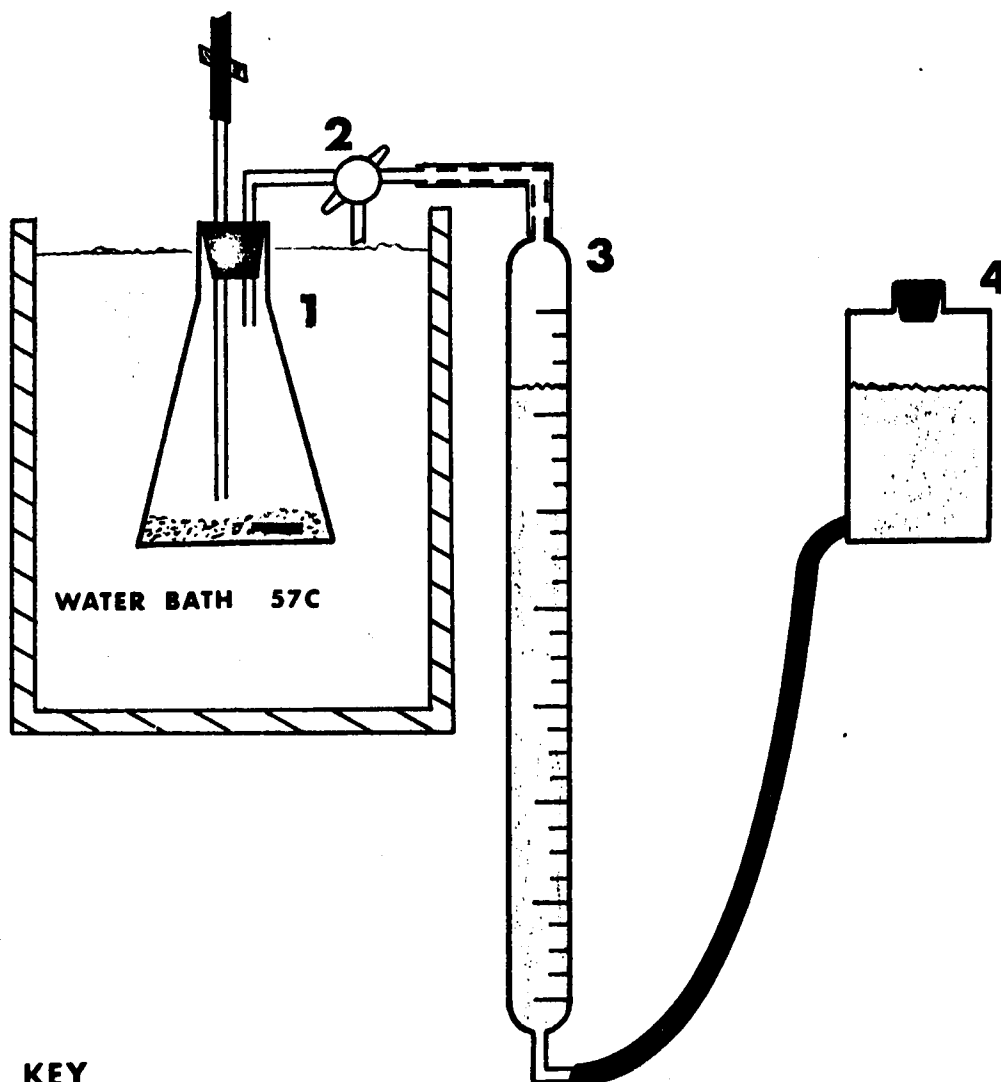
KEY

1. REACTION FLASK

2. 3 WAY STOPCOCK

3. ORSAT GAS BURET

4. LEVELING BOTTLE

===== GLASS TUBING ID. 1.75MM

█████ LATEX TUBING

===== GLASS TUBING IN LATEX

Figure 4. Assembly for Oxygen Uptake Determinations.

Jan. 1978

sized to fit a 24/40 joint, but in this instance employing a No. 3 - 2 hole rubber stopper which lodges securely inside the neck of the flask. A straight piece of heavy capillary glass tubing passes through one hole to within 20 mm. of the bottom of the flask while its upper end has a tight rubber sleeve and screw type clamp. A modified Orsat gas analysis manifold connects into the other hole of the stopper, its 3-way stopcock "2" located between the flask and the end connected by rubber sleeves and curved capillary tubing to the top of a 100 ml Orsat gas buret "3". The bottom of the buret connects by rubber tubing to an aspirator bottle "4", equipped with clamp for moving up and down alongside of the Orsat buret. A saturated solution of laboratory grade sodium chloride in distilled water is used to fill the Orsat buret and aspirator bottle assembly.

SAMPLE PREPARATION--Freshly produced press cake is ground twice employing a grinder plate with 1 cm diam. openings followed by one with 3.5 mm diam. openings. The ground scrap is divided into quantities needed for successive runs and transferred into plastic "boil-in" bags with low oxygen permeability. The insides of the bags and contents are steamed to elevate temperatures to about 100° C, then pressed tightly and sealed, frozen and stored at below -17° C.

Fish meal for O_2 uptake tests is prepared by placing the sealed plastic bag and contents in boiling water until the internal temperature is close to 100° C. While steaming the press cake is transferred to a chamber containing anhydrous calcium sulfate and held at 50° C for 12 hours while maintaining vacuum to reduce moisture content to 2 to 5%.

The press cake used so far has been made from small menhaden, producing fish meal with low fat content. To prepare samples for O_2 uptake determinations which do not require addition of menhaden oil a 10 gm. quantity of the vacuum dried scrap is ground in a glass mortar and pestle and promptly transferred into the reaction flask as a control. If tested in combination with another meal the combination is ground together in the mortar and pestle, vacuum dried to the 2 to 5% moisture range, then transferred to the reaction flask.

Alternately, for testing antioxidants which dissolve in menhaden oil, solutions of the antioxidants are prepared at concentrations which will deliver the required ppm level to 10 gm. of the freshly prepared vaccum dried fish meal upon addition and grinding in of 0.25 ml. of the oil. The control is prepared by combining untreated menhaden oil with the fish meal in the same ratio.

OPERATING PROTOCOL--In preparing for a test series the Orsat burets are completely filled with oxygen and the top ends clamped shut with the aspirator bottles supported in the 40 ml position.

Soda lime tubes plugged with fibre glass wool are placed in each of the oven dried reaction flasks. The samples are transferred to the flasks, stopper firmly inserted and then covered with thermoplastic coating (Pyseal). The flasks are sealed off from outside atmosphere by closing the clamp and stopcock until ready to introduce oxygen.

Introduction of oxygen into each flask and connecting to an Orsat buret is sequenced at 15 minute intervals. Oxygen is passed into the flask for 3 minutes via the straight capillary tube and leaves through the end of the manifold. At the end of three minutes the straight capillary tube is clamped shut and the manifold stopcock turned to seal off the flask while permitting free passage through the other two openings.

The clamp at the top of the buret is then removed and the end of the manifold connected firmly in contact with the curved glass capillary tube. While this operation is in progress the sodium chloride solution in the buret will rise to about a 50 ml level, thereby causing oxygen to replace the air in the connecting tubing. The stopcock is then turned to connect the buret to the reaction flask and the aspirator bottle positioned to arrive at equalization of inside and outside pressure, at which time the initial buret reading is taken.

The reaction flask is then swung down into the water bath and clamped in position with only a small part of the neck above water level. In all of the runs the water bath temperature has been held at 57° C, also noting ambient air temperature and pressure.

Expansion of oxygen in the reaction flask usually occurs in 15 minutes and is monitored by frequent positioning of the aspirator bottle. If such expansion is less than what is normally expected, then it is counted as part of the oxygen uptake of the first hour. A point of equilibrium occurs when expansion of the oxygen in the flask is completed after which further oxygen uptake by the sample is indicated by rising level of sodium chloride solution in the Orsat buret. Readings for a full 4 hour experiment are taken at 15 minute intervals.

RESULTS

These results must be considered tentative until further standardization and calibration is accomplished. Two examples are presented to indicate potential capabilities of using the equipment for checking stored fish scrap for residual antioxidant activity, and for screening various fish scrap stabilizing systems as basis for arriving at improved methods of storage.

ANTIOXIDANT ACTIVITY IN STORED FISH SCRAP--Ethoxyquin treated fish meal that was held in commercial storage for several months was vacuum dried and 0.25 ml. of untreated menhaden oil added to 10 gm. of the meal. Oxygen absorption was nil over a 4 hour period.

This same fish meal (i) was then compared with two other samples taken from commercial storage, representing (ii) very satisfactory storage conditions in large piles for six months, and (iii) unsatisfactory long term storage and evidence of oxidative deterioration.

The control for this experiment was 10 gms. freshly prepared vacuum dried fish meal while the samples of commercially stored fish scrap were vacuum dried and 5 gms. of each mixed with 10 gms. of the fresh vacuum dried fish meal.

Oxygen uptake in four hours, in ml./kg. based on amount of freshly prepared fish meal present in the sample was: Control = 4,870; (i) = 125; (ii) = 1,860; and (iii) = 2,560.

SCREENING ANTIOXIDANTS--This application was the basic reason for designing the equipment. It has enabled comparison of a number of antioxidants and of combinations of antioxidants with other ingredients. Figure 5. serves as an example. The control is typical of freshly prepared fish meal to which untreated menhaden oil has been added. The antioxidants were introduced by first preparing them dissolved in menhaden oil, the 0.25 ml aliquot to 10 gm of freshly prepared fish meal serving to introduce the correct ppm level of antioxidant.

The reactivity of the control and the performance of Antioxidant X in slowing down oxidation provides basis for examining its effectiveness in other ways. However before employing alternate methods the possibility of improving its performance by use of synergistic substances and with chelating agents can be explored as was done by introducing only 75 ppm of Antioxidant X, alone and with chelating agent.

Antioxidant Y, as shown in Figure 5., appears of doubtful value on the basis of poor performance in this test, but confirmation would rest on running several ppm levels.

DISCUSSION AND CONCLUSIONS

As explained there are many methods for determining how much oxygen enters a liquid, a moist, or a dry material, such approaches having been worked out and used for many years. A sampling of recent literature (1, 2, 4) indicates that it is still a valid approach.

During the mid-50's oxygen uptake measurements applied to fish scrap helped select antioxidants which were considered potentially useful for fish scrap. The results were found to relate to performance under processing plant conditions as opposed to the Active Oxygen Method which was less successful in predicting the outcome of practical applications.

An important result of antioxidant application to fish scrap and elimination of heating in storage was improved protein quality, increased metabolizable energy values, and less product variation. Lea, et al. (7, 8) and the research staffs of many feed manufacturing companies strongly emphasized the nutritional advantages of effective control of fish scrap oxidation.

The equipment, sample preparation and operating protocol discussed under METHODS AND MATERIALS appears to have two immediate applications. The first, dealing with antioxidant activity in stored fish scrap, requires refining of the technique in order to sharply define a situation where the protective action of the antioxidant no longer exists. It should be noted that Sample (iii) had suffered damage in storage, but still retained ability to reduce the oxygen uptake of the reactive fish meal with which it was mixed. Much guidance in working out a

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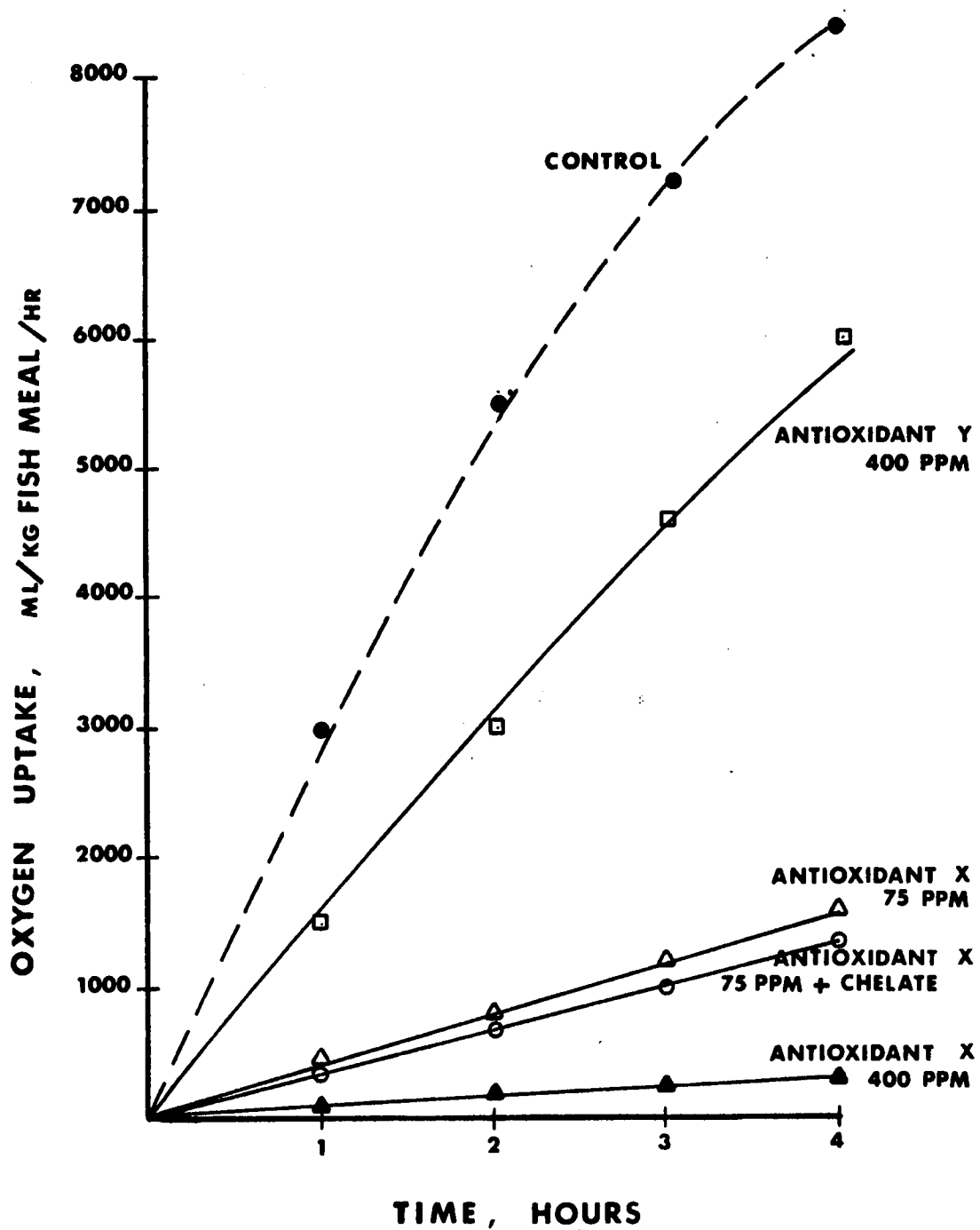
Figure 5. Oxygen Uptake by Fresh Fish Meal and
Menhaden Oil as a means of checking
Antioxidant Activity.

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suitable technique will result from consistent and organized checking of fish scrap at various recorded stages of storage, and the accumulation of sufficient data.

The protocol for screening antioxidants has been found to be capable of supplying quick results which can then provide basis for modifying the antioxidant system and seeking further improvements.

A number of other tests are useful in following oxidative changes and thereby evaluating antioxidant performance. It is beyond the scope of this paper to discuss such alternatives except to point out the capability of the equipment, discussed herein, to provide samples of fish meal oxidized under controlled conditions, which can then be subjected to such evaluations as total vs. ethyl ether extractable lipids, iodine value, free fatty acid, peroxide value and TBA.

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THE EFFECT OF AN EMPLOYEE EDUCATIONAL PROGRAM ON THE BACTERIOLOGICAL QUALITY AND YIELD OF BLUE CRAB PROCESSING

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Educational training programs for employees are useful in the food industry because they teach the employee the significance of proper sanitation. An increased awareness by the employee could increase not only personal hygiene practices but improve the overall plant condition. Present programs emphasize health and sanitation by showing films and demonstrating how personal hygiene could affect the quality of the product. The actual effects of sanitation workshops on the bacteriological quality of food have not been analyzed.

The blue crab industry was chosen for this study since employees personally contact blue crab meat at a critical point in processing. At this stage, destruction of pathogens is no longer possible without altering the flavor of the food. Cooking destroys most of the bacteria that were present on the live crab. However, extensive handling of crabs and crab meat reintroduces bacteria onto the crab meat, making the personal hygiene habits of employees the most important part of plant sanitation (1).

Industrial sanitary practices are related to the bacteriological quality of freshly picked blue crab meat (3,4,6). Insanitary conditions during the processing of food products are reflected by high aerobic plate counts (APC) and coliform organisms, and by the presence of indicator organisms such as $E.$ $coli$ and coagulase-positive staphylococci (8). With the analysis of bacteriological quality of crab meat, this study will evaluate possible short term effects from a health inspection and/or possible long term effects of a sanitation training program.

MATERIALS AND METHODS

Blue crab meat samples will eventually be collected from five Texas processing plants representing the following areas: Area 1 - Galveston Bay, Area 2 - Matagorda Bay, Area 3 - San Antonio Bay, Area 4 - Corpus Christi Bay, and Area 5 - Lower Laguna Madre.

The processing plants from these areas will be sampled by County Extension Marine Agents, trained by this investigator. Samples,

collected twice a week, will consist of 50 grams of special or flake crab meat. The crab meat will be analyzed for: APC, coliforms, E. coli, and coagulase-positive staphylococci.

The sample will be placed in a 450 ml sterile 0.1% peptone dilution. This initial dilution and subsequent dilutions will be used to prepare plates to determine APC, coliform, and staphylococci counts and to inoculate broth tubes to determine Most Probable Number (MPN) for coliforms, E. coli, and staphylococci.

After the marine agents have finished their initial inoculation, plates and tubes will be sent to College Station by commercial transportation. Once they arrive, the tests will be completed according to the Bacteriological Analytical Manual for Foods (5).

Baseline data will be obtained for four to five weeks. After the baseline data has been established, a health inspector will give a one-day inspection. Additional data will be collected for a two-week period; then employees will participate in a sanitation workshop. After the workshop, data will be collected for another four-week period.

The workshop will be similar to the Seafood Quality Control (2) pamphlet. This exclusive program for crab plants will be composed of a 45-minute film followed by a 45-minute instruction.

RESULTS

The crab plants were slow in opening up this season, therefore, only preliminary results from two areas can be given. Table 1 shows the initial baseline data from Area 5 - Lower Laguna Madre and Area 3 - San Antonio Bay.

Table 1. Preliminary Results. Ranges of bacteriological counts are collected from Area 5 and Area 3.

Area 5 (6 samples)

APC	1.6×10^4 org/gm - 4.5×10^5 org/gm
Coliforms - MPN	43/gm - 1,100/gm
E. coli	< 3/gm
staphylococci	< 10/gm - 160/gm Direct Method
	9.1/gm - 43/gm MPN

Area 3 (4 samples)

APC	5×10^4 org/gm - 5.4×10^5 org/gm
Coliforms - MPN	23/gm - 43/gm
E. coli	< 3/gm
staphylococci	< 10/gm - 400/gm Direct Method
	3.6/gm - 23/gm MPN

DISCUSSION

As of now, since no conclusions can be drawn from the data, only objectives will be stated. Future results will be analyzed for possible short term effects from the health inspector and/or long term effects of the sanitation training program. These effects will be analyzed by statistical decreases in the bacteriological levels of crab meat. Also, any changes in bacteriological levels will be compared with possible changes in yield and productivity of crab meat. An employee's new awareness of sanitation could lower his working capacity and thus the yield of crab meat.

The bacteriological levels will also be compared with differing plant sanitation. The sanitation gradings of the processing plants will be obtained from the Department of Health Resources, Division of Shellfish Sanitation. Based on these gradings, the plants will be ranked from one to five on overall sanitation and compared to bacteriological test results.

The last objective compares the two different methods of direct plating and Most Probable Number (MPN) to detect staphylococci and coliforms. According to the Bacteriological Analytical Manual (7), the number of staphylococci may be obtained by direct plating of Baird Parker agar or by inoculating into broth tubes for the MPN technique. In addition, Violet Red Bile agar plating for coliforms is used routinely by many dairy and poultry processing plants as short cuts around the more laborious MPN. The different methods will be compared for effectiveness and practicality.

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EFFECTS OF SAMPLING PROCEDURES ON
SALMONELLA RECOVERY FROM FRESH WATER CATFISH

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Salmonellae is only rarely reported in fresh water or marine species of fish (1,3,10,11,13,14), and is then generally associated with fecally contaminated water. Testing for salmonellae is usually initiated by either swabbing (4,12,16), rinsing (7,8,21) or blending (2,22) a sample in pre-enrichment or selective enrichment broths. A recent study of salmonellae detection on dressed frog legs by blending, immersion of whole legs, maceration with a Stomacher and by rinsing showed no significant difference in recovery for the first 3 methods, whereas, rinsing was significantly inferior (2).

MATERIALS AND METHODS

In this study, 350 samples from 200 frozen catfish known to be contaminated with salmonellae were tested for the presence of salmonellae by various sampling procedures. Method I consisted of dividing a fish in half by severing it just behind the anal vent. The tail portion (posterior-IP) and the visceral portion (anterior-IA) were then weighed and blended separately in 9 parts of lactose broth (LB). One-half of each homogenate was incubated at 35°C for 24 hours and the remaining halves were combined and reblended for homogeneity. This composite sample was then halved and one-half incubated at 35°C for 24 hours (composite-IC). The other half of the composite homogenate was incubated at 43°C for 24 hours (composite-IC43). Method IC served as the control method for this study. Method II consisted of complete immersion of the entire fish in LB. Method III included shaking the entire fish in LB for 1 minute and removing it prior to incubation. Method IV consisted of swabbing the entire surface area of the fish using 2 dacron swabs moistened with LB and placing them in 20-ml LB. The LB pre-enrichment for Methods II, III and IV were incubated at 35°C for 24 hours. Ten samples were initiated daily for each method. Pre-enrichment was

followed by the selective enrichment of 1-ml portions of LB in 10-ml tetrathionate (TET) and 10-ml selenite cystine broth (SEL). Brilliant green agar with sulfadiazine, bismuth sulfite agar and Salmonella-Shigella agar with 1% sucrose and 0.65% agar added as recommended by Sperber and Deibel (20) were streaked from TET and SEL. Suspect salmonellae colonies as described in the BAM manual (22) were picked from each plate to triple sugar iron agar slants and motility-indol-lysine (MIL) deeps. MIL is a modification of Ederer and Clark's (5) motility-indol-ornithine medium (MIO) with lysine substituted for ornithine. MIL was prepared by adding 1% trypticase and 0.2% agar to Falkow lysine broth. This medium allowed for the biochemical differentiation of salmonellae from indol-positive Edwardsiella. Incubations were at 35°C for 24 hours at each step of the isolation procedure. Suspect salmonellae isolates were further tested for Methyl Red, Voges Proskauer reactions, utilization of citrate on Simmon's citrate agar and in 0.5% mannitol in purple broth base for acid production. Serological confirmation of salmonella was performed with Poly-O-antisera using the slide agglutination method. All media used in this study was BBL with the exception of SS agar from Difco.

RESULTS AND DISCUSSION

The percentage of salmonellae positive samples is given in Table 1. An analysis of variance was performed to determine if there was a difference in the recovery rate of the methods. The F value obtained indicated that a significant difference did exist between the methods tested. Duncan's new multiple range test was performed to determine which methods were significantly different. The methods underscored and joined by a solid line had no significant difference. Those not connected by a line are significantly different. Method IC43 had the highest recovery rate, but was not significantly different from Method II. This indicates that the effectiveness of the increased incubation temperature recommended by several workers (5,6,15,17,18,20) is also applicable for salmonellae detection in catfish. No significant difference was established between Method II, Method IA and the control Method IC. The control method was based on the requirements in the BAM manual (22) which calls for a 25-g portion from an unspecified area of the fish to be blended in 9 parts of LB and then incubated at 35°C. This study indicated that a significant difference existed between the presence of salmonellae in the visceral cavity and the tail area of catfish. This could have an effect on the salmonellae recovery rate if indiscriminate selections are made on portions of the fish to be tested for salmonellae. The higher incidence of salmonellae in the visceral cavity can be expected because of increased chances of contamination from the visceral and from increased handling during processing. The presence of salmonellae on the tail area would have to be a direct result of cross-contamination during processing.

Based on these results the recommended procedure for maximum recovery of salmonellae from catfish would be immersion of the whole fish or visceral cavity of large fish in LB followed by incubation of the inoculated media at 43°C. According to statistical analysis, the increased temperature was not significantly better than Method II,

	+	%	-	TOTAL
IA - Anterior portion	19	(38)	31	50
IP - Posterior portion	13	(26)	37	50
IC - Composite sample	19	(38)	31	50
IC43- Composite at 43°C	25	(50)	25	50
II - Whole fish	21	(42)	29	50
III - Rinse	7	(14)	43	50
IV - Swab	7	(14)	43	50

IV   III   IP   IA   IC   II   IC43

TABLE 1.   Percentage salmonellae positives by different
recovery methods and the relative significance
of each method.

although it did have the highest recovery rate. Method II probably
has more value as an isolation method because of the simplicity
in sample preparation and the minimal equipment needed for incubation.
The sample can be collected and pre-enriched in the same poly bag,
and incubated in a dry-heat incubator; whereas, a water bath is re-
quired for incubation at 43°C to maintain a constant temperature.

A study in sample methodology by the FDA on salmonellae iso-
lation was in general agreement with the results of this study (2).
The methods they compared were immersion of whole frog legs, blend-
ing use of the Stomacher and rinsing. They found no significant
difference in the first three methods, but recovery was significant-
ly lower with rinsing. They did not use 43°C as an incubation tem-
perature.

Methods IA, IP, IC and IC43 had a total of 41 (82%) salmon-
ellae positive fish of the 50 fish tested. Ideally a salmonellae
positive IA or IP sample would have the corresponding IC and IC43
sample positive. However, this only occurred in 11 of 19 IA
positives and 9 of 13 IP positives. This indicates that only 20
(62.5%) of the 32 IA and IP positive samples had sufficient num-
bers of salmonellae to be recovered in the corresponding composite
sample. Only 13 IC positives corresponded to the 25 IC43 positives.
The IC43 method had the highest recovery with 25 positives, but
it missed 16 (33.3%) of the total 41 salmonellae positive fish.
This indicates that although a large percentage (82%) of the pop-
ulation was salmonellae positive that the number of salmonellae
present on each fish was at a low level. In addition, the poor
recovery (14%) obtained with contact methods (rinse and swab) in-
dicates that very few salmonellae are dislodged from the surface
area of the fish.

The purpose of preventing salmonellae contamination of raw
food products such as catfish, poultry and frog legs is to prevent
cross-contamination of a food that does not receive a terminal
heat treatment. The poor recovery by contact methods and the low
numbers indicated by Method I positives does not support catfish as
a potential salmonellae hazard. The FDA recently affirmed this pri-
marily based on their 1977 study (1), but theorized that it was
because of the processing procedure used to process catfish. The
procedure they described was developed by Dr. Ranzell Nickelson and
myself (23) and was only in operation at one plant. The FDA was
aware of the procedure because it was a part of a complete sani-
tation program submitted to them for approval so the plant could
resume processing after being enjoined to stop processing by the
FDA. This procedure was proven to be effective in producing sal-
monellae-free carcasses. However, additional research (24) has
shown that salmonellae in live fish is directly related to the level
of salmonellae in the water. Salmonellae was found to be continu-
ously present at low numbers, but under the proper conditions of
temperature, stocking rate and fish body weight the number of sal-
monellae in the water rapidly increased. The presence of salmon-
ellae on the skin and in the viscera of live fish resulted in sal-
monellae-positive carcasses under normal processing procedures

used to dress catfish. The findings of this work found salmonellae
to be present at a high frequency (82%) on a specific lot of fish,
but that they were present at low numbers. Additional findings
showed that the presence of salmonellae (24) on catfish was de-
pendent on certain variables, and that a procedure is available
to preclude its presence on the finished carcass (23). This fur-
ther reinforces the low potential of catfish as a salmonellae
hazard.

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PRELIMINARY REPORT ON
THE USE OF A SPECIFIC-ION ELECTRODE (AMMONIA)
IN DETERMINING THE QUALITY OF SHRIMP

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Numerous methods have been investigated by the shrimp indus-
try for the rapid and accurate determination of shrimp quality;
however, none are being used. Currently, the industry lacks the
ability to recognize the degree of freshness of raw shrimp. This
inability to consistently recognize shrimp of varying quality has
several ramifications. Penalties in the form of lower prices can-
not be imposed for poorly handled catches, nor can bonuses be payed
for high quality, well handled product. Additionally, marketing
judgements with respect to the final disposition of product are
difficult. In other words, in the retail marketing of fresh shrimp,
shrimp with the highest quality will have the longest shelf-life,
therefore, proving advantageous not only to the retailer but also
the consumer. On the other hand, shrimp of acceptable quality,
but possibly not the highest, would be better utilized in frozen
products.

The purpose of this study was to evaluate the use of a specific-
ion electrode to measure the ammonia content of shrimp held under
iced and non-iced but refrigerated storage conditions. Addition-
ally, direct microscopic counts (DMC) were made and correlated with
ammonia production.

The use of a specific-ion electrode in determining the quality
of fresh seafoods has been reported by Chang, et al. (1976) who
developed a trimethylamine specific-ion electrode for the purpose
of rapidly evaluating fish quality. The choice of ammonia as an
indicator of quality degradation in shrimp is significant in light
of the work by Vanderzant, et al. (1973) which indicated that ammo-
nia accounted for a large percentage of the total volatile nitrogen
production during shrimp spoilage.

MATERIALS AND METHODS

Penaeus setiferus (white) and Penaeus duorarum (pink) shrimp
were headed with shell remaining prior to placement in ice for trans-
port to the Food Quality and Safety Laboratory. Samples of white
shrimp were run daily for the first ten days and every other day for
another ten days. Pink shrimp samples were run every other day.

Three shrimp were weighed in sterile whirl-pak bags. Sterile 0.1% peptone buffer was added to give 1:2 weight to volume dilution. The sample was shaken vigorously and 0.01 ml was transferred using a precalibrated metal syringe onto a glass slide with 1 square centimeter areas delineated. Slides were air dried at 35°C for 30 minutes. Slides were Gram stained, examined under oil immersion, and the DMC's enumerated according to Standard Methods for the Examination of Dairy Products (1972) and Nickelson et al. (1975.)

For ammonia analysis, 10 ml of the sample was pipetted into a beaker and 40 ml of distilled water added to make a total volume of 50 ml. Using an Orion ammonia specific-ion electrode coupled into a Corning model-110 digital pH meter, millivolt readings were obtained while agitating the solution with a teflon coated magnetic stirring bar and pipetting 1 ml of 10N NaOH into the solution. The NaOH drives ammonia ions (NH_4 +) to ammonia (NH_3), a gas, for which the membrane of the electrode is permeable. Ammonia concentrations were determined from a standard curve plotting millivolt readings versus known concentrations of ammonia chloride.

RESULTS AND DISCUSSION

Figure 1 indicates the relationship of ammonia production and bacterial numbers of white shrimp stored over time under various conditions. Non-iced shrimp were taken from the iced shrimp after 9 days of storage and refrigerated. The graph shows that bacterial counts increased dramatically over the 20 day storage period in the iced shrimp and, as would be expected, even more dramatically in the non-iced condition. Ammonia production in iced shrimp also increased with time, although values did not consistently increase from one day to the next, the trend was increased ammonia concentration with time of storage. This observation is particularly true with non-iced shrimp, which produced increased ammonia concentrations with time of storage, presumably due to the absence of the washing effect of the melting ice.

Figure 2 is a plot of the log number of bacteria vs. the log concentration of ammonia. The data indicates a very high degree of correlation between bacteria numbers and ammonia production. The correlation for iced and non-iced white shrimp is 0.86 and 0.94 respectively. Nickelson et al. (1975) demonstrated that DMC's on green-headless shrimp could be correlated with agar plate counts (APC) incubated at 25°C. The researchers indicated that DMC values were a log higher than corresponding APC values.

A second study was undertaken to determine the ability to correlate bacterial numbers and ammonia production in a different species of shrimp. Figure 3 indicates that the degree of correlation for iced and non-iced pink shrimp is 0.86 and 0.97 respectively.

CONCLUSION

In summary, the authors would like to indicate that there may

be some potential in utilizing this technique for the evaluation of shrimp quality, however, it is not ready for industrial application. Variables such as other species of shrimp should be investigated. Additionally, procedures need to be modified to make use by industry more practical. Nonetheless, it appears that increases in bacterial populations and time of storage correlate with ammonia production which may have possibilities in rapidly determining the quality of raw products with an ammonia specific-ion electrode.

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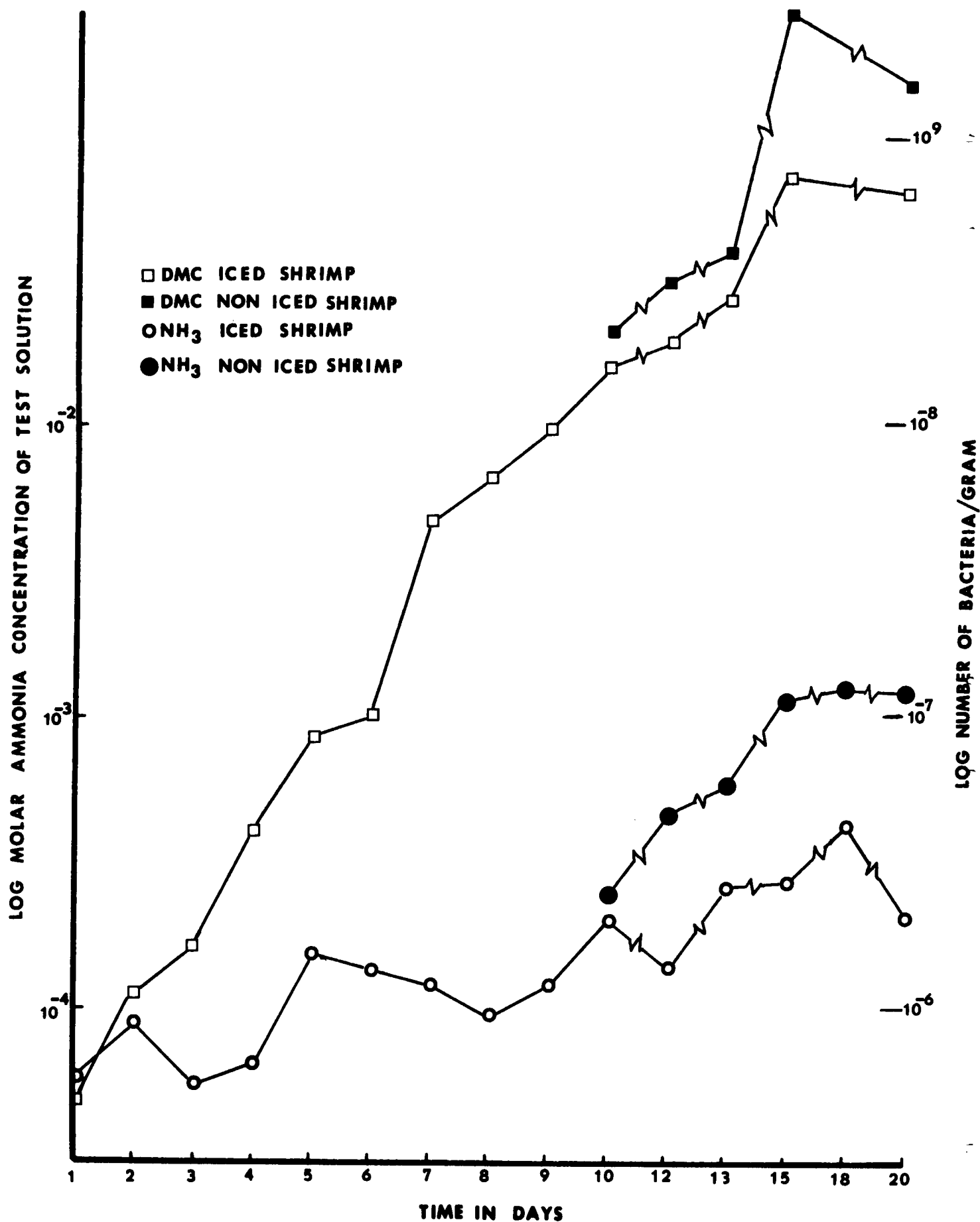
FIGURE 1 - Ammonia concentration and direct microscopic count in white shrimp during iced and non-iced storage.

FIGURE 2 - Relationship between annonia concentration and direct micro-
scopic count in white shrimp during iced and non-iced storage.
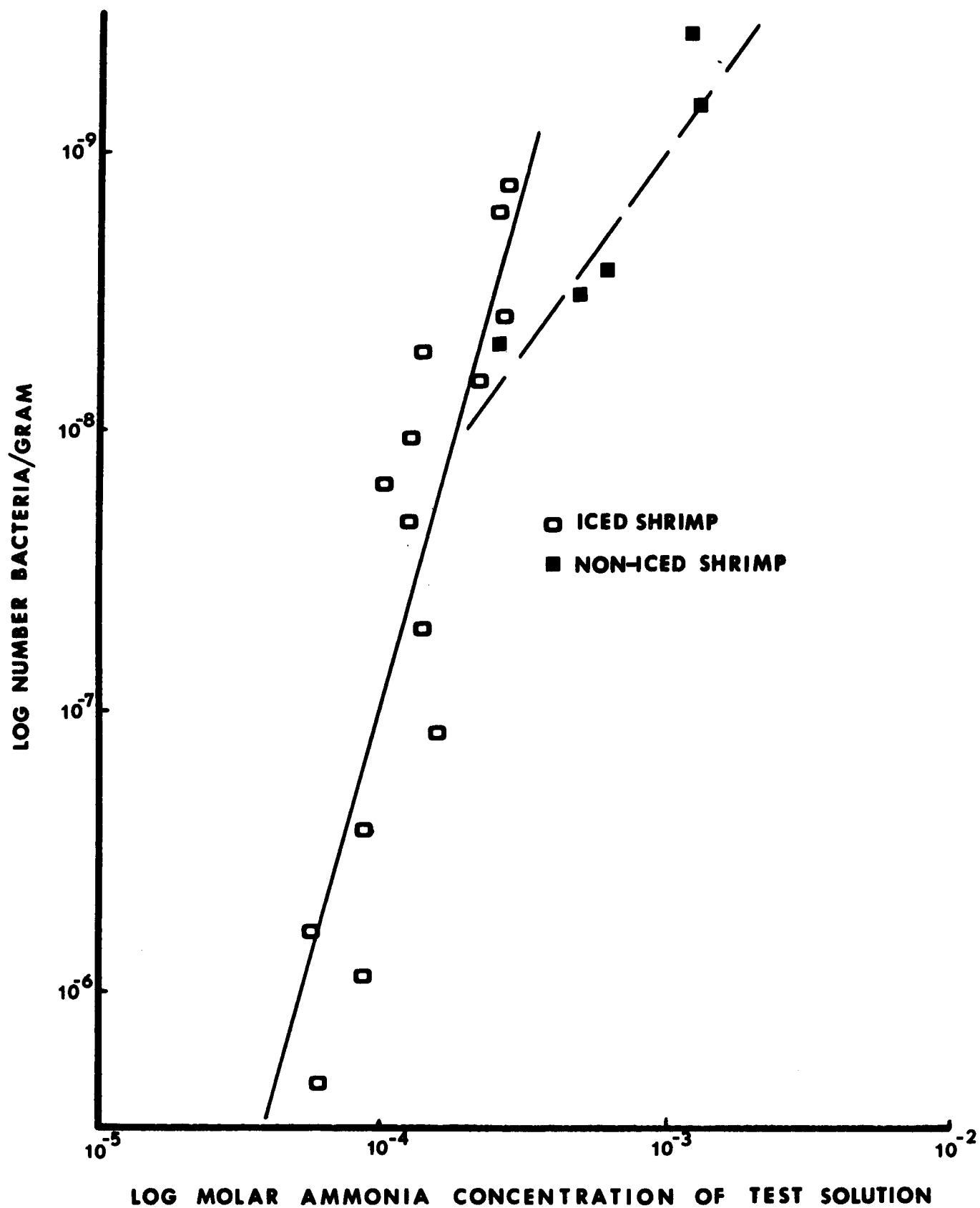
EVALUATION OF THE AGAR PLATE PROCEDURE FOR IMViC TYPING OF FECAL COLIFORMS ISOLATED FROM SEAFOODS

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The presence of coliforms in processed foods has historically been used to indicate post sanitation and post processing contamination. The coliform procedure is designed to detect all lactose fermenting members of the Enterobactericeae group, some of which have little sanitary significance. Therefore, a recent trend has been the utilization of the fecal coliform test because it is more specific for Escherichia coli, an organism definitely linked to fecal contamination. It is recognized that the fecal coliform test is not completely specific for E. coli and thus has its limitations. In instances when it is desirable to know if E. coli is present, the IMViC procedure for separating different coliform types must be utilized.

The conventional IMViC procedure is time consuming, requiring at least 96 hours to complete after the coliforms are isolated in pure culture and inoculated into the IMViC media. Powers and Latt (3) realizing the shortcomings of the conventional procedure, developed a modified IMViC procedure which both produced results comparable to the conventional procedure and reduced the incubation time to 48 hours. The modified procedure employs solid media in "X" -compartmented petri dishes instead of liquid media in tubes. Parallel testing of coliform types demonstrated the accuracy of the new procedure. However, these workers realized that for the 48-hour agar plate IMViC procedure to become widely accepted, it must be tested by several laboratories using coliforms isolated from a variety of sources.

The purpose of the work reported here was to evaluate the 48-hour agar plate procedure for IMViC typing of fecal coliforms isolated from seafoods.

MATERIALS AND METHODS

All oyster samples examined were fresh raw oysters from approved areas, opened in a processing plant or in the laboratory. Crabmeat samples represented meat obtained during various stages of processing in the plant. In all, 27 oyster and 31 crabmeat samples were employed in this study.

Homogenization and inoculation of the samples into lauryl tryptose broth (LTB) was in accordance with accepted procedures (1, 2). All gassing LTB tubes were inoculated into EC medium and incubated at 44.5C for detection of fecal coliforms. EC positive tubes were streaked onto Eosin Methylene Blue (EMB) plates and incubated at 35C. A single typical coliform colony on each EMB plate was selected for IMViC typing by both procedures.

For the modified IMViC agar plate method, growth was picked from the selected colony and inoculated directly onto the IMViC agar plate media (3). After a 48-hour incubation at 35C, the plates were read as directed by Powers and Latt (3) with one exception, that being the Kovac's solution was prepared with butanol rather than isoamyl alcohol (4).

Conventional IMViC typing followed procedures outlined in APHA (1) in which the selected colonies were first inoculated onto agar slants and incubated for 18-24 hours before Gram staining and inoculation into the conventional tubed IMViC media. At this time, the cultures were also inoculated into LTB to confirm them as coliforms. Any cultures showing discrepancies in the IMViC pattern between the two tests were restreaked on EMB agar and retested by both procedures.

RESULTS AND DISCUSSION

A total of 479 fecal coliform cultures isolated from raw oysters and processed crabmeat were IMViC typed by both the conventional and the 48-hour plate procedure. Identical IMViC patterns were obtained with 96.7% of the cultures. Table 1 shows the different IMViC patterns displayed by isolates from both types of seafood products. Isolates which displayed the same IMViC pattern with both test procedures are listed as comparable and those isolates which did not are listed as uncomparable. Little difference was noted in the percent of uncomparable isolates from the two sources.

Table 2 displays the IMViC patterns as determined by both procedures for the 16 cultures listed as uncomparable. No discrepancies were noted in the citrate reaction and

only two in the indole reaction. Of the remaining discrepancies six occured in the MR reaction, five in the VP reaction, and three in both the MR and VP reactions.

Five of the cultures which showed discrepanices on the MR reaction only were both MR and VP positive when tested by the tube procedure. Powers and Latt (3) found that the methyl red reaction of some known cultures remained falsely positive in the MR-VP broth, whereas the reaction on the plates was as expected. Therefore, in these cultures, the nonconformity may have been the result of an error in the tube test rather than the plate procedure.

Occasionally, when reagents were added to the VP portion of the plate, it became cloudy and difficult to read. This may have produced the discrepancies with the five cultures showing different VP reactions between the two procedures. The reason for the remainder of the discrepancies is unknown.

False-positive E. coli IMViC patterns (++-- or -+--) were not observed with the plate, and only three cultures produced false negative patterns (+++-) as compared to the tube procedure which showed a ++-- pattern. Since discrepancies rarely occured with the indole and citrate portions of the test, cultures showing the +++- IMViC reaction on the plate procedure should be considered as possibly false-negative E. coli and be retyped by the tube procedure.

Early in our use of the plate procedure, we experienced poor resolution in the color reactions on the MR-VP portion of the test. The problem was traced to the plastic petri dishes which had been stored in the laboratory for approximately eight years. When we switched to the Falcon brand dishes as specified by Powers and Latt (3) these problems dissipated. Powers (personal communication) indicated he had experienced a similar problem when using a brand of petri dish other than Falcon.

After having tested the 48-hour agar plate IMViC typing procedure in our laboratory over an eight-month period, we feel the procedure is accurate and has an advantage over the tube procedure in that it is time saving in three steps. First, 24 hours can be saved by inoculating the IMViC media directly from the EMB plates. Second, 48 hours are saved in the incubation time for the IMViC media. Lastly, we have found the plate method affords at least a 25% time savings in media preparation, inoculation, reading test results and clean up. We encourage other researchers to test the agar plate IMViC procedure for use in their laboratories.

ACKNOWLEDGMENT

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Table 1. IMViC reaction of fecal coliform isolated from seafoods and typed by both the agar plate and conventional tube procedures.

| | Cultures Isolated From | | | | | |
| | Crabmeat | | Oysters | | Overall | |
	Number	Percent	Number	Percent	Number	Percent
Comparable[a]						
++--	30	24.4	152	42.7	182	38.0
-+--	-	-	2	0.6	2	0.4
--++	88	71.5	175	49.2	263	54.9
+-++	-	-	5	1.4	5	1.1
---+	1	0.8	5	1.4	6	1.3
-+-+	-	-	3	0.8	3	0.6
++-+	1	0.8	-	-	1	0.2
+++-	-	-	1	0.3	1	0.2
Total Comparable	120	97.5	343	96.4	463	96.7
Uncomparable[b]	3	2.5	13	3.6	16	3.3
Totals	123	100.0	356	100.0	479	100.0

a - Cultures producing the same IMViC pattern when typed by both procedures.
b - Cultures producing different IMViC patterns when typed by both procedures.

Table 2. IMViC reactions of fecal coliform isolates which were uncomparable when tested using the agar plate and conventional tube procedure.

Tube IMViC Reaction	Plate IMViC Reaction	Discrepency	Number of Isolates
++--	+++-	VP	3
+-++	--++	Indole	1
--++	+-++	Indole	1
-+-+	--++	MR-VP	3
++++	+-++	MR	2
-+++	--++	MR	3
-+-+	-+++	VP	1
--++	---+	VP	1
+-++	++++	MR	1

EFFECT OF BOILING, FRYING, MICROWAVE HEATING AND CANNING ON THE PROXIMATE, MINERAL AND THIAMIN CONTENT OF SHRIMP

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Although the nutritional composition of fresh shrimp is well documented (2,13), little information exists on the effect of cooking method on nutrition retention. Reber (12) found the percent protein in boiled shrimp to decrease on a dry weight basis. Many cooking conditions above 60°C are reported to cause a shrinkage in fish protein with concomitant loss of liquid containing water-soluble components (10). While large losses of minerals are reported during cooking of various foods (8), Korobkina (6) noted that losses of phosphorus, copper and other minerals from heat processed squid could be reduced by shortening the length of heat treatment. Although Srinivas et al. (9) found no differences in ash, phosphorus, and calcium between fresh and canned shrimp, Watt and Merrill (13) did report higher amounts of ash, calcium, phosphorus and iron in canned shrimp. In addition, thiamin was reduced 50% in shrimp (13) and 75% in canned swordfish (11). The purpose of this study was to determine the effect of boiling, microwave heating, canning, and frying on the proximate, mineral and thiamin content of shrimp.

MATERIALS AND METHODS

Materials

Medium size fresh brown shrimp (Peneaus aztecus) obtained from Apalachicola, Florida, were used in this study. The freshly caught shrimp were placed in large plastic bags at the wharf, iced and immediately transported to the laboratory in Gainesville, where they were washed and deheaded. The samples were stored at -30°C until processed and analyzed.

Cooking Methods

Boiling

Shrimp were thawed for 90 minutes at room temperature, placed in a stainless steel container with three times the shrimp weight of

boiling water, and boiled for five minutes. After 10 minutes cooling, the shrimp were peeled, ground twice through a food grinder and analyzed.

Frying

Shrimp were thawed for 90 minutes at room temperature and peeled. Shrimp were dipped in batter prepared by mixing one cup ice water, 1 egg, 1/2 teaspoon sugar, 1/2 teaspoon salt, 1 cup all purpose sifted flour and 1/4 cup soybean oil. The shrimp were fried eight at a time for three minutes at 190°C in a Sunbeam Deep Fryer (Model CF-5) containing one gallon Swift's refined soybean oil. After frying, the batter was removed by hand from the shrimp and the shrimp were passed twice through a food grinder, then analyzed.

Canning

Shrimp were thawed for 90 minutes at room temperature and peeled. The shrimp were parboiled for four minutes in a two-gallon steam jacketed kettle containing one gallon boiling 9% NaCl brine. After draining, the shrimp were packed in 307 x 113 lacquer coated cans, covered to capacity with hot (65°C) 1% NaCl brine and sealed. The cans were processed for 15 minutes at 121°C in a 55 gallon capacity still retort. After cooling, the cans were opened and the shrimp analyzed.

Microwave cooking

The shrimp were thawed 90 minutes at room temperature and cooked eight at a time in a Litton Menumaster Systems 70/50 Microwave Oven (2450 Mhz). After cooking for 30 seconds and allowing 15 seconds for heat distribution with the oven off, the shrimp were recooked for 30 seconds. The shrimp were then peeled, ground twice through a food grinder, and analyzed.

Chemical Analysis

Moisture analysis

Ground shrimp were analyzed in triplicate for moisture content according to AOAC (1). Approximately 10 grams of shrimp were added to two grams of asbestos fiber and 5 ml of distilled water in 50 mm x 40 mm aluminum pans and dried overnight at 100°C in a forced air oven.

Crude fat analysis

Crude fat was determined by a modification of the AOAC method (1). Approximately two grams of dried shrimp was placed in a 100 ml Mojoinnier flask and solubilized by heating with 8 ml concentrated hydrochloric acid on a steam bath for 60 minutes. After cooling, 10 ml of 95% ethanol and 25 ml of ethyl ether were added. After shaking, 25 ml of petroleum ether was added, the flask was reshaken and then centrifuged at 600 RPM for 10 minutes. The fat layer was decanted into a

beaker and the portion remaining in the Mojoinnier flask was extracted twice again with 15 ml of ethyl ether and 15 ml of petroleum ether which was added to the beaker. The sample was then evaporated to dryness and dried further at 100°C for 90 minutes.

Protein analysis

Dried shrimp were analyzed for protein by the AOAC standard microKjeldahl method (1). The factor 6.061 was used to convert nitrogen to protein since it is more appropriate for fish flesh than the common factor 6.25 (5).

Ash analysis

Ash was determined by heating approximately three grams of dried shrimp overnight in a muffle furnace slowly brought to 550°C.

Mineral analysis

Ash was dispersed with 2 ml deionized water and heated at 100°C for 1 1/2 hours and then reashed as described above. Approximately 200 mg of ash was then dissolved in 50 ml of 0.2 N hydrochloric acid. Calcium, cobalt, copper, chromium, iron, magnesium, manganese, nickel, strontium, and zinc were analyzed by atomic absorption spectroscopy. Potassium and sodium were determined by flame emission spectroscopy and phosphorus was determined colorimetrically (4).

Thiamin analysis

Thiamin was determined using the thiochrome method of Mickelson and Yamamoto (7).

Statistical analysis

The data was subjected to analysis of variance and Duncan's multiple range test (3).

RESULTS AND DISCUSSION

The proximate composition of fresh and cooked shrimp is shown in Table 1. As expected, moisture content was reduced by all cooking procedures. Fried shrimp were lowest in moisture since there is no way of replenishing water lost during frying. Microwave heated shrimp had more water than fried shrimp and although water was not added to microwave heated shrimp, the shorter heating time probably helps to account for the higher water content. On a wet "as eaten' basis, protein content was inversely proportional to moisture content in cooked shrimp and apparently increased in all cooking methods. Fried shrimp were highest in protein on a wet basis but lowest in protein on a dry basis due to the high amount of fat present. Fried shrimp contained the most fat due to the use of oil during cooking. While there was no

difference in fat content between boiled, canned, or microwave heated shrimp on a wet basis, microwave heated shrimp did contain less fat on a dry basis. Boiled shrimp had the least ash while canned shrimp had the most. Since no salt was added to the boiling water, minerals were leached from the shrimp during cooking. Since canned shrimp were processed in salt brine, some of the minerals leached out were replaced with salt taken up by the shrimp from the brine.

The caloric content of shrimp increased with all cooking methods (Table 1). Fried shrimp were highest in kilocalories due to the high amount of fat present. Kilocalories were calculated by multiplying grams of carbohydrate, fat, and protein by the factors 4 Kcal/g, 9 Kcal/g, and 4 Kcal/g, respectively.

Canned shrimp had the highest percent ash and sodium content but was lowest in phosphorus, potassium, magnesium and iron content (Table 2). The high sodium content of canned shrimp is probably related to the use of salt brine in the processing. Boiled shrimp were lowest in sodium content due to the osmotic leaching effect of the boil water. All heat processed shrimp had more calcium than fresh shrimp. This is most likely related to intracellular binding of calcium which prevents it being leached during the heat processing. The difference in iron content between boiled and canned shrimp is of interest since it was expected that the leaching effect would be the same in both methods. Evidently, the iron content of canned shrimp is diluted by the sodium taken up during processing and appears to be less. No differences were detected for cooked or fresh shrimp in zinc, strontium, cobalt, or nickel.

Heat processing tends to increase the nutritive contribution of the average serving of shrimp for most minerals (Table 3). Microwave heated and fried shrimp contained more thiamin than fresh shrimp on a wet basis (Table 1). This is related to the lower moisture content of the cooked samples. Boiled and canned shrimp contained 20% and 80% less thiamin respectively than fresh shrimp. Heat lability, cooking time and leaching of the water-soluble vitamin by the cooking water are most likely responsible. On a dry basis, however, thiamin content decreased in all cooked samples relative to length of cooking time. This decrease in thiamin content is similar to thiamin losses observed in canned swordfish (11).

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% (wet basis)

	Fresh	Boiled	Microwave Heated	Canned	Fried
Moisture	77.6+0.2a	73.3+0.6b	70.4+1.0c	73.2+0.1b	62.1+1.1d
Protein (Nx6.061)	19.6+0.0a	23.9+0.2b	26.1+0.9c	22.7+0.3d	27.3+1.1e
Fat	0.9+0.0a	1.3+0.0b	1.3+0.0b	1.3+0.0b	6.5+0.5c
Ash	1.8+0.1a	1.6+0.1b	2.3+0.1c	2.6+0.2d	2.4+0.1e

% (dry basis)

	Fresh	Boiled	Microwave Heated	Canned	Fried
Protein (Nx6.061)	87.2+1.6a	89.1+1.4b	88.2+1.5ab	84.4+1.1c	71.7+1.7d
Fat	4.0+0.2a	4.8+0.1b	4.2+0.1c	4.8+0.1b	17.1+0.8d
Ash	8.0+0.3a	6.1+0.3b	7.6+0.3c	9.8+0.6d	6.2+0.1b

mg thiamin per 100 grams of shrimp

	Fresh	Microwave (1 min)	Fried (3 min)	Boiled (5 min)	Canned (20 min)
Wet basis	0.05+0.0a	0.06+0.01b	0.06+0.0b	0.04+0.0c	0.01+0.01d
Dry basis	0.23+0.01a	0.19+0.01b	0.16+0.01c	0.14+0.01d	0.06+0.01e

Kcal per 100 grams of shrimp

	Fresh	Boiled	Microwave Heated	Canned	Fried
Kcal	87	107	116	103	175

Means + Standard Deviation

Values followed by same letter are not statistically significant (P<0.05).

Table 1. Effect of Cooking Method on Proximate Composition, Thiamin, and Caloric Content of Shrimp.

mg/100 g shrimp flesh

	Fresh	Boiled	Microwave Heated	Canned	Fried
Phosphorus	269+11a	251+31a	329+10b	226+14c	358+16d
Sodium	238+41a	178+36b	280+49c	709+114d	428+27e
Potassium	167+49a	158+38a	260+65b	76+30c	165+83a
Calcium	94+9a	129+8b	125+12b	126+16b	132+11b
Magnesium	46+3a	54+5b	60+7c	50+3d	64+10e
Iron	2.2+0.4a	2.8+0.5b	3.0+0.3b	1.8+0.3c	2.5+0.3a
Zinc	1.3+0.2a	1.7+0.3a	1.7+0.4a	1.7+0.3a	1.9+0.6a
Strontium	0.32+0.05a	0.46+0.12a	0.50+0.14a	0.40+0.04a	0.47+0.11a
Copper	0.33+0.03a	0.42+0.03b	0.46+0.02c	0.33+0.07a	0.41+0.05b
Manganese	0.08+0.01a	0.09+0.02ab	0.11+0.02c	0.10+0.01bc	0.10+0.01bc
Chromium	0.04+0.04a	0.03+0.02a	0.03+0.02a	0.05+0.04a	0.08+0.04a
Nickel	0.01+0.02a	0a	0a	0a	0.03+0.06a
Cobalt	0a	0a	0a	0a	0a

Mean + Standard Deviation

Values followed by the same letter are not statistically significant ($P < 0.05$)

Table 2. Effect of Cooking Method on Mineral Composition of Shrimp

	RDA(mg)	Fresh	Boiled	Microwave Heated	Canned	Fried
Phosphorus	1200	22.4	20.9	27.4	18.8	29.8
Magnesium	400	11.1	13.5	15.0	12.5	16.0
Iron	18	12.2	15.5	16.6	10.0	13.9
Zinc	15	8.7	11.3	11.3	11.3	12.7
Calcium	1200	7.8	10.8	10.4	10.5	11.0

[1]RDA for 18 year old male equivalent to USRDA
Food and Nutrition Board, National Academy of Sciences
Recommended Dietary Allowances, 1973

Table 3.   Percent Recommended Dietary Allowances[1] for Minerals
           from 100 Grams of Shrimp Cooked by Different Methods

MICROBIAL DEVELOPMENT ON SHRIMP AS AFFECTED
BY DELAYED HEADING

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Economic pressure on the shrimp industry has caused a change in the practice of heading all shrimp immediately upon harvest. Increased crew wages and limited space for handling shrimp aboard ship during periods of peak harvest have resulted in less heading aboard ship. Consequently more shrimp with the heads on are entering processing plants and the retail market. In addition, the potential development of a market utilizing shrimp heads for specialty chemical products would probably require deheading of shrimp on shore. Shrimp heads are a potential source of pigments, chitin, chitosan, fatty acids, protein and flavor compounds (5). Another important factor is that the retail price of shrimp can be kept to a minimum when they are sold with the heads still on.

This trend toward landing shrimp with heads on stresses the need for information as to the effect of this practice on shrimp quality. Therefore, this study was conducted to determine the effect of delayed heading on microbial growth and flora changes during 10 days of ice storage.

MATERIALS AND METHODS

Samples: A total of five samples of shrimp were obtained, two from the West Coast and three from the East Coast of Florida. Approximately 20-30 lbs of fresh shrimp were obtained at dockside, packed in ice, and transported to the laboratory in Gainesville, Florida. The shrimp were rinsed with tap water and half of each shipment was headed before storage. The tails and whole shrimp were then rinsed in cold tap water and packed in ice in separate styrofoam containers. After 5 days of ice storage the remainder of the shrimp were headed and returned to storage. All shrimp were medium size (36-50 count) and were mixed species of the genus Penaeus.

MICROBIOLOGICAL METHODS

Samples were drawn for analyses at 0, 5 and 10 days. Pour plates were prepared with Standard Plate Count agar (SPC) containing 0.5% salt using 1 ml aliquots of the buffered homogenate (1,2). Duplicate SPC plates were incubated at 20°C for 5 days.

Tails stored separately: Three 50-g subsamples of shrimp tails were drawn at 0, 5 and 10 days and homogenized in 450 g of Butterfield's buffered dilution water (2) for two minutes in a Waring Blendor. Serial dilutions were prepared and inoculated into SPC agar plates.

Delay headed shrimp: On the fifth day of storage, shrimp stored with heads on were headed and returned to ice for an additional five days. Because of the large variability in bacterial counts encountered in shrimp, a sample of 10 shrimp with heads on was drawn at each sampling period (0 and 5 days). Each of the 10 shrimp was then headed and analyzed.

Isolation and identification of microbial flora: After total plate count determinations, a number of colonies equal to the square root of the number of colonies on the countable plates were picked and isolated for identification. Colonies were picked at each sampling period from all treatments. A total of 516 random colonies were obtained. Standard microbiological methods were used throughout (23), except that 0.5% salt was added to all media used for identification purposes. Identification was according to the description in the 8th Ed. of Bergey's Manual of Determinative Bacteriology (4) and A Guide to the Identification of the Genera of Bacteria (22).

RESULTS AND DISCUSSION

There are only a limited number of reports on the effect of heading shrimp on microbial quality. Green (12) observed that the head accounts for only 32-40% of the total weight of shrimp, yet contains 50-80% of the bacterial load. She also states that the intestinal tract which contains some bacteria is usually removed with the head and therefore, bacterial populations tend to multiply more rapidly in head on shrimp than on shrimp stored with heads off. To increase the storage life of fresh shrimp, she recommends heading and washing before icing, and washing again when unloading and re-icing. In 1951, Lantz (19) stated that removal of the head extended the storage life of uncooked shrimp by 2 days and reduced the degree of discoloration. He added that the heads contain a large number of bacteria, which can be transmitted to the surface of the tails where they invade the tissue. Cann et al. (7) stated that unless Nephrops species can be landed live, heading at sea is essential in order to prevent detrimental autolytic changes resulting from enzymes, particularly those present in the digestive glands.

However, recent studies by Bieler et al. (3) and Koburger et al. (15), have shown that heading might not be necessary under all conditions. Bieler et al. (3) reported that rock shrimp stored with heads on had lower total counts, maintained higher solids content and greater organoleptic acceptability than did tails stored alone. In 1973, Koburger et al. (15) reported that Penaeus shrimp contained essentially the same total counts whether the shrimp tails were stored with or without heads. They also found that a 15-member taste panel could not detect a significant difference in flavor, texture or overall acceptability between shrimp stored with or without heads throughout a 14-day storage period.

Figure 1 shows the average bacterial counts of shrimp tails stored without heads and those delay headed after 5 days, through 10 days of ice storage. In the five studies, fresh shrimp tails count ranged from 7.5 x 10^5 to 2.5 x 10^6 bacteria/gram. Although these initial counts seem high, they are within the ranges reported by other investigators (6,12,8,10,24,9). In addition, these counts are lower than the geometric mean of the aerobic plate count of fresh shrimp reported by Foster et al. (11). As shown in Figure 1, bacterial growth on shrimp tails proceeded at the same rate in both treatments. At the 10th day of storage, shrimp stored without heads had an average bacterial count of 9.0 x 10^8 bacteria/gram while delay headed shrimp tails contained 1.2 x 10^9 bacteria/gram. Thus, indicating little difference in counts due to the different treatment.

Twenty-two genera and 38 species of bacteria were found on fresh shrimp with five genera predominating, Flavobacterium, Pseudomonas, Vibrio/Aeromonas group, Moraxella and Planococcus (Figure 2). These bacterial types are similar to those found by Campbell and Williams (6), Koburger et al. (14), and Lee and Pfeifer (17) on shrimp and similar to the types found in the slime of many marine fish (20).

A change in the frequency of isolation of the six major bacterial groups was observed on the fifth day of ice storage (Figure 2). Flavobacterium, Moraxella and Vibrio/Aeromonas still predominated and Pseudomonas and Planococcus were found to decrease slightly. This change was observed in shrimp stored both with and without heads.

Following 10 days, Pseudomonas and Planococcus were the predominant groups found. No major differences were found in the percentages of the various groups of organisms found in shrimp tails stored without heads and those delay headed at the tenth day. Thus, delay heading does not markedly affect bacterial composition and proliferation in shrimp tails. Not only the same bacterial groups predominate but they are also present in approximately the same percentages.

Interestingly, Flavobacterium predominated for the first five days of ice storage then decreased. Pseudomonas isolates decreased until the fifth day, then increased rapidly. Other workers have observed the presence of Flavobacterium in raw shrimp (6,13,14,17,24, 25). They also noted the decrease in numbers of Flavobacterium after ice storage with an increase in Pseudomonas species. Pseudomonas species have been implicated by Shewan (20), Shewan et al. (21) and Shaw and Shewan (19) as the organisms primarily responsible for spoilage of marine fish stored in ice. Figure 3 shows this shift in bacterial flora during ice storage of shrimp tails and delay headed shrimp.

Other organisms isolated from shrimp subjected to both storage treatments were Bacillus, Micrococcus, Acinetobacter, Azotobacter, Alcaligenes, Proteus and Cytophaga in order of decreasing occurrence.

Thus, similar bacterial growth patterns and bacterial flora were observed in shrimp tails subjected to the two different storage treatments. Therefore, it appears that the practice of not heading shrimp immediately following harvest will not markedly affect the quality of the shrimp as long as the shrimp are stored properly for a reasonable time. In addition, when prolonged ice storage is anticipated, leaving the shrimp intact may serve to prevent exposure of the surface tissue to proteolytic organisms in the slime layers and also confines the intestinal contents (21).

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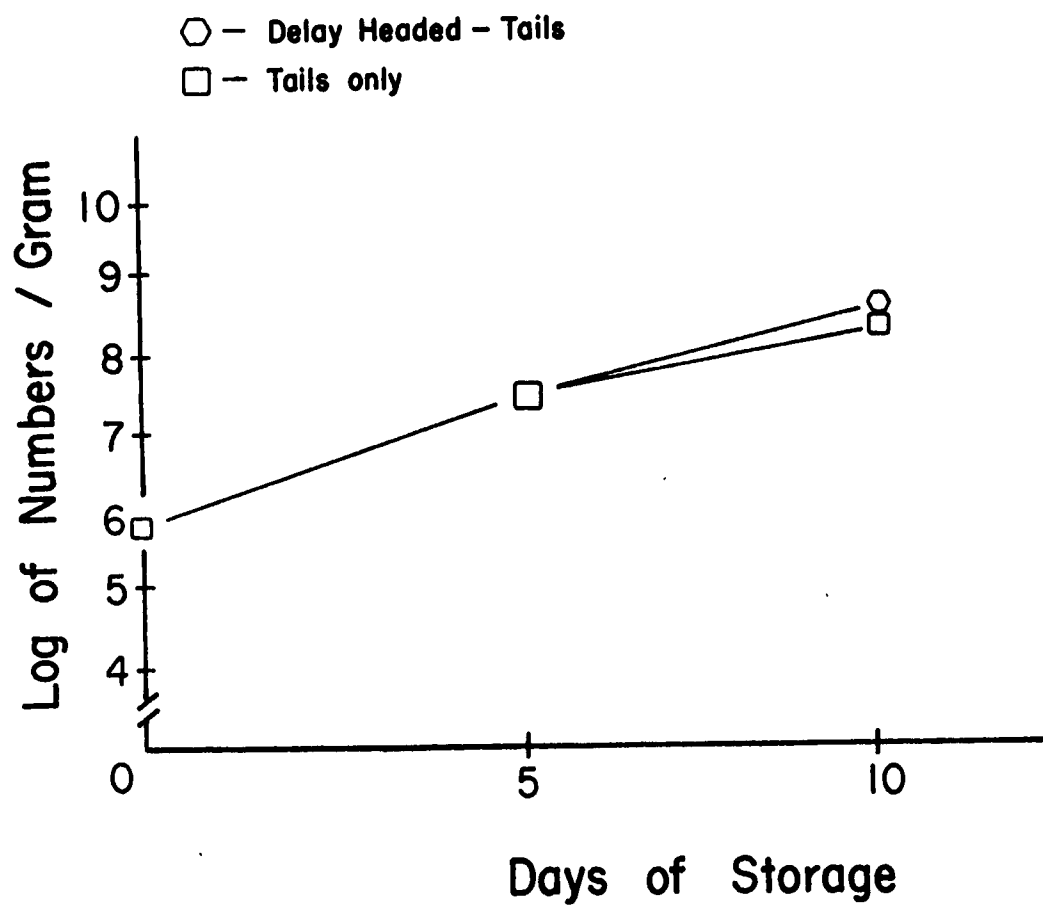
FIG. 1. AEROBIC PLATE COUNT OF STORED SHRIMP
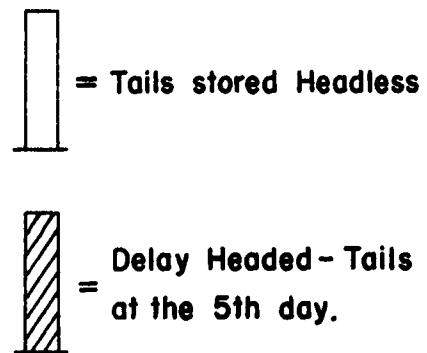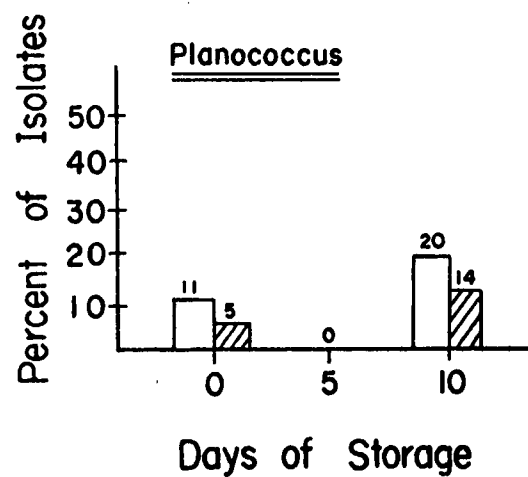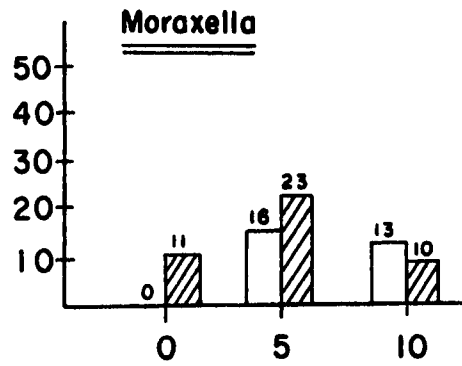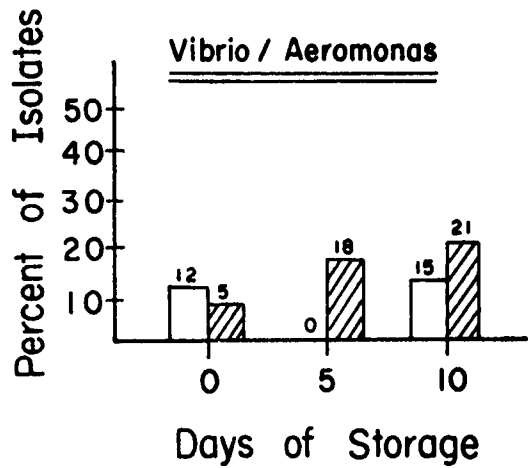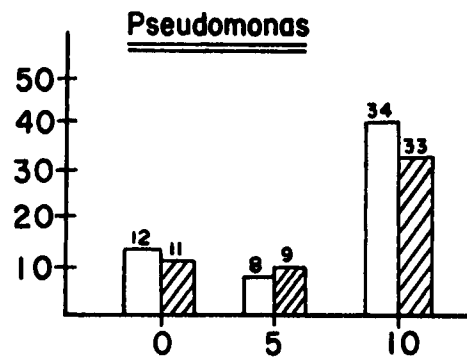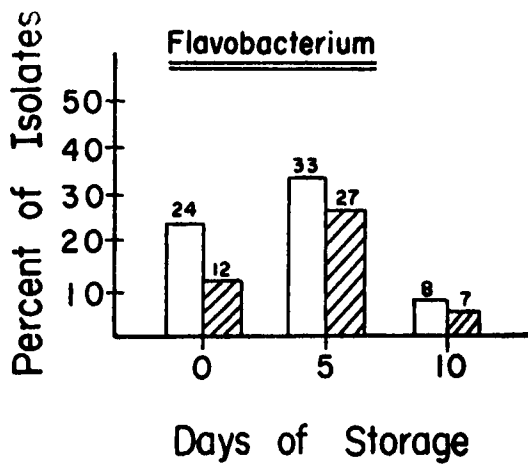
FIG. 2. FREQUENCY OF ISOLATION OF THE MAJOR BACTERIAL GROUPS RECOVERED FROM SHRIMP TAILS STORED WITHOUT HEADS AND DELAY HEADED.
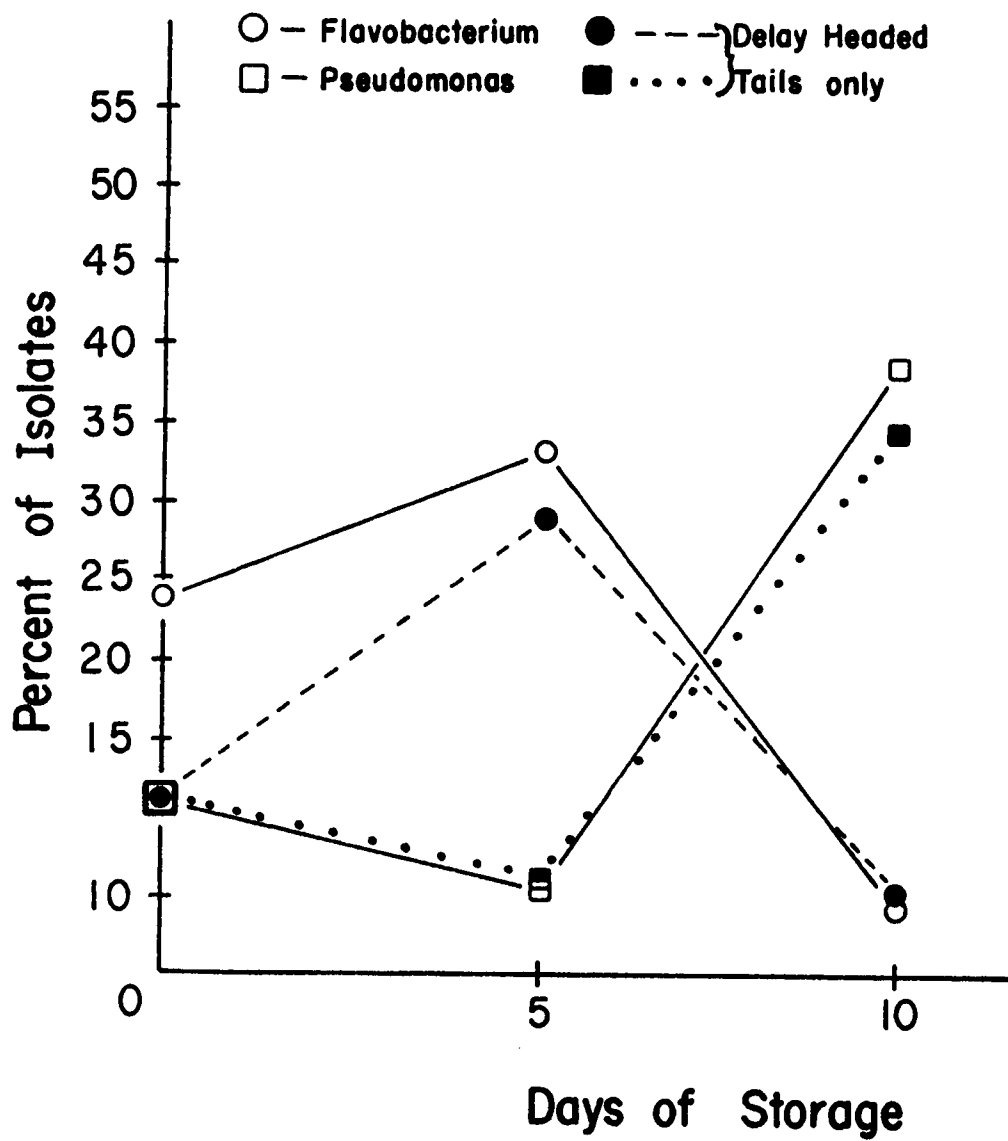
FIG. 3. CHANGE IN BACTERIAL FLORA DURING ICED STORED SHRIMP

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OBJECTIVE COLOR DETERMINATION
AS A QUALITY INDEX FOR
PANULIRUS ARGUS

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The current price of Florida Spiny Lobster tails at the retail market in Florida is now $10.00 per pound. These premium prices would seem to imply premium quality, however, the demand is so great and the supply so limited that often tails of poor quality are offered for sale.

The origin of the product is the entire Caribbean area. The methods employed in harvesting and preservation vary from primitive to modern. Improper handling by the fisherman is the primary cause for degradation of the product. It is therefore most important to assess quality at the point of transfer from fisherman to processor.

Most processors purchase lobster tails in a lot which usually comprises the entire catch of one boat. Depending on the treatment given the catch and the amount of time involved there may be considerable variations in quality within one lot. Due to this variation random sampling plans may give an inaccurate assessment of the entire lot.

In the normal processing of lobster tails a processing step known as grading is performed, which is simply segregating the tails into weight classes. It is at this point that a scale operator can utilize a visual test to sort for quality. Implementation of this test at this point would generate a number of tails that failed the visual test, these could then be further examined by chemical or organoleptic means by a technician that could not possibly examine each tail personally.

Current military specifications require that the tail be free of "any discoloration." Codex Alinorm 78/18A requires that the tail be "practically free of blackening or other abnormal discoloration." Due to the biological variation in P. argus color of shell and tissue is not consistant in all populations. In order for a guideline or standard to be useful the term "abnornal discoloration" must be carefully defined.

J. J. Ryan 1971 noted that a standard should be developed in accordance with a general set of principles:

1. The standard should be based on the actual styles, types and quality gradations of the product being produced and marketed.

2. The standards should be national in scope.

3. Standards should take into consideration the important factors that affect the relative desirability of the product.

4. Standards must provide for options, or personal choices, with regard to style, type and species.

5. Standards require the proper selection of factors upon which quality is to be based so that the factors can be appropriately and consistently applied.

6. Standards should incorporate whenever possible, objective tests and procedures for use in determining compliance.

7. Standards shall be prepared to achieve quality evaluation within an economically reasonable inspection time period and cost.

The proposed color evaluation is not meant to be the sole basis for a standard on lobster tails, however, as part of a guideline or standard it must meet the above criteria.

In the selection of the actual test method or procedure additional criteria need to be satisfied. Shewan and Jones (1957) noted the following requirements for an objective index of quality:

1. It should be present or absent in constant amount in the freshly caught fish.

2. It should accumulate or disappear quickly and at a steady rate during spoilage.

3. It should be capable of being simply and speedily estimated.

MATERIALS AND METHODS

This method utilizes a KODAK "gray scale" which is a photographic standard consisting of ten tones of gray each representing an 18% decrease in reflectance. (Figure 1.) This scale is used as a reference to quantify the amount of "black spot" or melanosis on the ventral surface of the tail. The card can be placed on the weighing platform and a quick visual calculation can be made by comparing the tones and counting the number of segments affected. By having the color evaluated by the scale operator the sorting of the lot can be accomplished with little change to the normal processing procedure.

By defining abnormal discoloration as grayness exceeding .70 in two or more segments, any tails that exhibit this amount or more would be selected for additional testing.

The photographs in the paper were made with KODAK PLUS X 35mm film and were printed on KODAK POLYCONTRAST RC paper.

RESULTS AND DISCUSSION

Selection of factors to be evaluated for the determination of quality and consumer acceptance must encompass the desires of the ultimate consumer. In seafood marketing the visual appearance of the product is of great importance especially when the products are packaged in sealed containers.

Lobster tails that appear normal from the dorsal aspect and from the appearance of the meat where it has been separated from the cephalothorax may often exhibit extensive discoloration and/or deterioration when examined from the ventral aspect. It was noted that the discoloration occured first in the thinnest posterior segments of the tail, the discoloration becomes more intense and affects the anterior segments under the following conditions:

Insufficient icing, improper washing/deveining, and aging of the product. In the normal fresh condition the ventral surface remains clear.

Samples that showed grayness exceeding .70 in two or more segments were examined were found to exhibit the following characteristics:

a. Samples were found to lack the characteristic sweet taste typical of the species. Samples exceeding .70 in four or more segments were found to exhibit off flavors.

b. Amoniacal odors were noted only in samples showing discoloration in all segments. All other samples were found to have normal odor.

c. Samples with four or more segments affected were judged to have texture defects as they were lacking in firmness of the muscle tissue.

CONCLUSIONS

The amount of melanosis exhibited on the ventral surface of the Spiny Lobster tail is the most useful visual aid in the determination of product quality.

A continuous spectrum of color change occurs in this area and is related to the other quality factors such as taste, odor and texture.

In products that are individually handled, an evaluation of discoloration if used as a guideline, would provide the processor with an effective tool for quality assurance.

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Figure 1
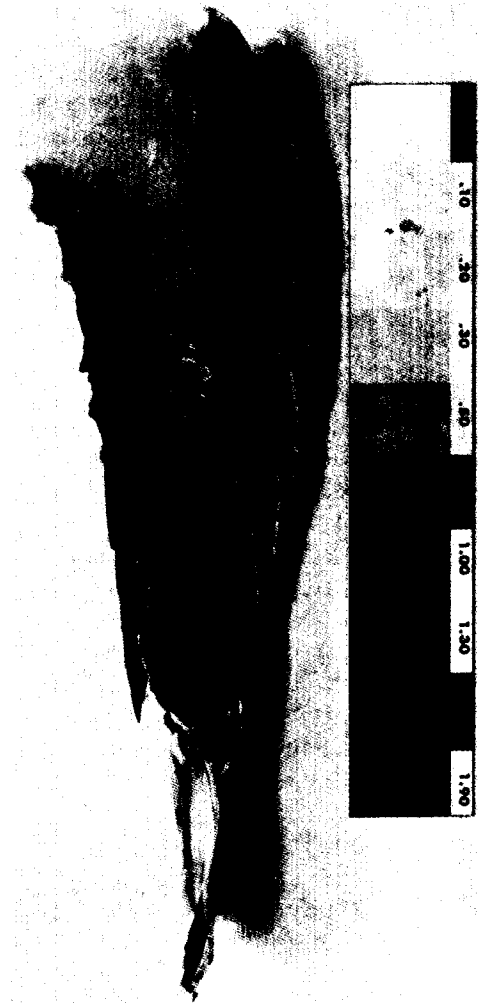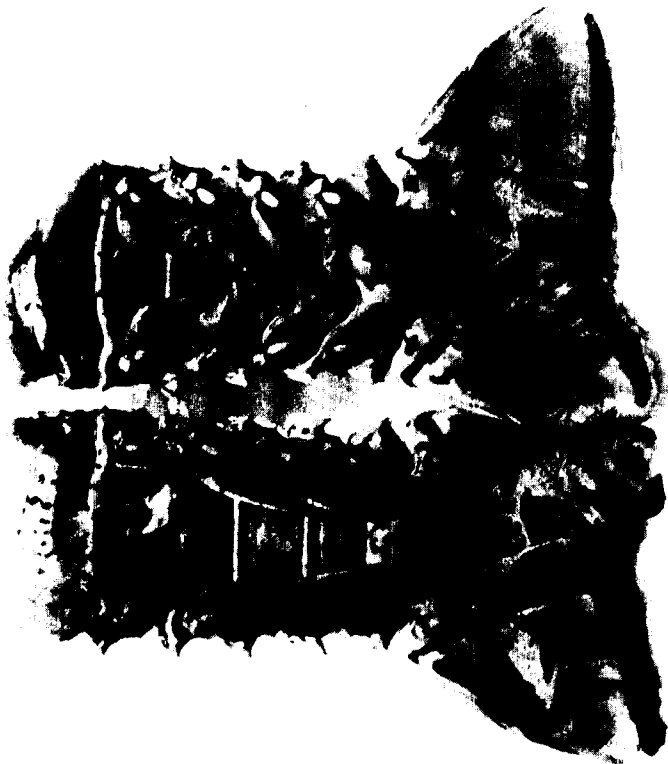
OYSTER DRIP LOSS
PART I: VARIABILITY OF DRIP LOSS IN
COMMERCIALLY SHUCKED OYSTERS FROM GULF STATES

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For many years there has been a desire among regulatory agencies, consumer groups and industry members to establish a standard or guideline to govern drip loss of fresh shucked oysters. A nationally-agreed upon standard has not been developed (with the exception of the Food and Drug Administration's Standard of Identity for Raw Shucked Oysters) because a practicable maximum drip loss tolerance could not be agreed upon. The FDA Standard of Identity allows 5 percent weight loss when the oysters are examined within 15 minutes after packing.

Representatives of some of the oyster-producing states along the east coast feel that a maximum 10 percent drip loss by weight should be adequate during storage of the product. Gulf coast producers maintain that this percentage is too low, and that such a restriction would result in harsh economic consequences, including plant shutdowns. Gulf producers insist that the southern oyster is subjected to differing environmental conditions from that of the Chesapeake Bay oyster, and further point out that southern oysters can reasonably be expected to exhibit an 18 percent drip loss during chilled storage of the product. The point of contention of producers of southern oysters is that their raw material--having been grown in a warmer environment and subjected to greater fluctuating temperatures and salinities--exhibits different drip loss characteristics during chilled storage.

Several studies have been conducted in an effort to find an equitable solution to the excessive free liquor problem confronting the southern oyster industry. Novak et al. (5), based on a 5-year study of the free liquor content of Gulf oysters, proposed to change the free liquor content, measured 15 minutes after packing, from 5 to 10 percent in the Federal definitions and standards for shellfish as applied to oysters. They found, however, that in many instances their samples exceeded their own proposed 10 percent free liquor content.

Another study on the drip loss behavior of shucked oysters during processing and chilled storage was conducted by a team of scientists known as the Government-Industry Cooperative Oyster Research (GICOR) Team. Results of this study were published in the Journal of the Association of Agricultural Chemists (3, 4). The authors showed that shucked southern oysters when permitted to bleed freely

lost almost half their weight in 24 hours, with more than half that loss occurring within the first few minutes of shucking. Fieger et al. (2) in another study reported similar results.

Additionally, over the intervening years, government-industry teams have shown that when skimmer-drained oysters are permitted to absorb fresh salt-free water, they re-absorb more than half their weight during 24 hours, but only one-fourth of this gain occurs during the first half hour of surface/volume contact time. These researchers concluded that the extent and rate of drip loss of un-washed Gulf oysters were identical to that of Atlantic oysters even through the fresh water uptake during washing is greater for Gulf oysters. Based on these results, it is reasonable to assume that for southern oysters most of the fresh water uptake during draining and subsequent storage is lost as free liquor.

In view of all the data published indicating that southern oysters are different in their handling and storage characteristics, it is disconcerting that no positive action has yet been taken toward resolution of the problem in applying Federal regulations on an equitable basis to oysters from differing geographical locations. The FDA, for example, historically has refused to promul-gate a dual standard for shucked oysters from different geographi-cal locations. A practicable and uniform standard should be promulgated that would allow southern oyster producers to market their product on a more economically competitive basis.

In an attempt to assist in resolving this dilemma, the Shell-fish Institute of North America requested the National Marine Fisheries Service to obtain baseline data on the drip loss of Gulf-produced shucked oysters. Recognizing the usefulness of this type information, plans were developed to obtain the data which could form the basis for a uniform national standard.

The objectives of the study were twofold: (i) to conduct a 1-year study to gather baseline data from commercial production from four Gulf states, and (ii) to determine if Gulf producers could meet the 10 percent drip loss tolerance suggested by the aforementioned research, and, if not, to recommend a percentage that would be practical and not severely punitive.

MATERIAL AND METHODS

A flow sheet describing the plan of work is shown in Figure 1. Sampling began in March 1970 and terminated in April 1971. The sampling protocol consisted of obtaining samples of commercially shucked oysters (6 pints and 3 gallons) monthly from four states (Florida, Mississippi, Alabama, and Louisiana), from a single dealer in each state practicing various methods of processing. The shellstock from commercial shucking was always obtained from the same location in the Gulf of Mexico in order to negate environ-mental variations. Processed samples were collected by an analyst at the plant immediately after commercial shucking and packing, and packed in ice and transported to the Pascagoula laboratory. All samples were analyzed for drip loss 2 hours after being packed for shipment. After the initial 2-hour examination, the samples (including liquor removed during analysis) were chill-stored at 4°C for 14 days and again subjected to drip loss analysis. The

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14-day time period was selected to parallel the recommended chilled storage time for oysters at the retail level. In addition to drip loss determination, all samples were analyzed for salt, moisture, and pH.

The above-described analyses were repeated each month except when it was impossible to obtain samples due to the size of the oysters, adverse weather conditions, pollution, etc. All analyses were performed in accordance with the standard methodology as described in the AOAC Manual (1).

RESULTS AND DISCUSSION

Results of the year's study on commercially shucked oysters are shown in Figures 2, 3, 4, and 5.

Each value for pints represents an average of 6. The data indicates that drip loss from pints (Figs. 2, 3), analyzed 2 hours after packing, ranged from 0 to 21 percent, averaging 9 percent. Drip loss from the pint samples stored 14 days ranged from 5 to 34 percent, averaging 18 percent.

Each value for gallons represents an average of 3. On initial examination, drip loss from gallons (Figs. 4, 5) ranged from 4 to 23 percent, averaging 9 percent (similar to the product packed in pint containers). Drip loss for gallons stored for 14 days ranged from 10 to 38 percent, averaging 21 percent (slightly more than that for the pint containers).

As expected in a study dealing with a multiplicity of environmental influences, the results were erratic. There was little correlation shown between areas from which the samples were taken and time of year the samples were taken. Additionally, the data fluctuated considerably between months of harvest. Consequently, no consistent pattern was evident. A notable exception to the foregoing was that all samples (pints and gallons) reached a maximum drip loss in September or October, and a minimum during November, January, or March. In an earlier study, Novak et al. (5) concluded that March was the only month that Gulf oysters could meet the FDA Standard of Identity for a 5 percent loss, and our results agree with such findings.

The drip loss variations between pints and gallons were comparable, with a slightly greater loss indicated for gallons, probably due to differing container sizes with the gallons having greater pressure on the bottom meats. In both container sizes, drip loss from the 14-day stored samples paralled those of the initially examined samples. A greater drip loss was exhibited from the 14-day stored samples from Florida and Alabama than those from Louisiana and Mississippi. Such can probably be attributed to the commercial practice in Florida and Alabama of blowing the oysters prior to packing. Currently, state laws in Mississippi and Louisiana prohibit that commercial cleaning practice.

The extreme variability of data between harvesting areas and months of harvest is probably due to the physiological differences of the oysters and continual changes in their biological environment (i.e., temperatures, salinities, etc.).

Review of Figures 2, 3, 4, and 5 reveals that commercially shucked oysters from the states included in this study could probably meet

the 10 percent drip loss tolerance shortly after shucking, but only under carefully controlled processing conditions. Should the tolerance be applied after prolonged chilled storage, however, the product would require a drip loss tolerance of 15 to 18 percent, and as much as 20 percent for product produced in those states permitting the blowing method.

Salt and moisture analyses were conducted concurrently with those for drip loss to determine any correlation. As would be expected, there was an inverse relationship between the salt and moisture content--oysters containing high levels of moisture had reduced salt levels, indicating prolonged contact with fresh water either before or after shucking.

The pH did not change appreciable between months of harvest. Results did indicate that the pH averaged 6.25 for the initial analysis, and dropped to an average of 5.75 after 14 days of chilled storage.

A limited study was conducted to determine the effect of the commercial practice of blowing Gulf-produced oysters. Observations from this study were: (i) oysters increased in weight an average of 6.7 percent due to the blowing process, (ii) a small number of the samples of blown oysters retained more liquor than the non-blown controls after 14 days of storage indicating less drip loss, and (iii) salt content decreased in the blown oysters indicating fresh water uptake by the oysters. These conclusions were as expected. No explanation can be offered for the retention of liquor by the small percentage of blown oysters during chilled storage other than such could be due to the physiology of the oyster.

In summary, the oyster is a very delicate animal that is composed of 80 to 85 percent moisture and approximately 1 percent salt when shucked. When the environment of the shucked oyster deviates from the normal physiological state of the shellstock even slightly, profound changes take place in the shucked meats during chilled storage. Also, the stress that oyster shellstock undergoes due to changing water conditions will determine to a great degree the drip loss in commercially shucked oyster meats.

To illustrate this point, oyster shellstock was taken from both the eastern and western areas of Apalachicola Bay in Florida on the same day. The oysters were shucked and the accompanying drip loss was measured during chilled storage. The results showed that oysters taken from the eastern area (where the tide fluctuates) were small and lean, and exhibited considerable drip loss. The oysters taken from the western area (where the tide fluctuates very little and exposure to fresh water is minimal) were large and fat, and produced very little drip loss.

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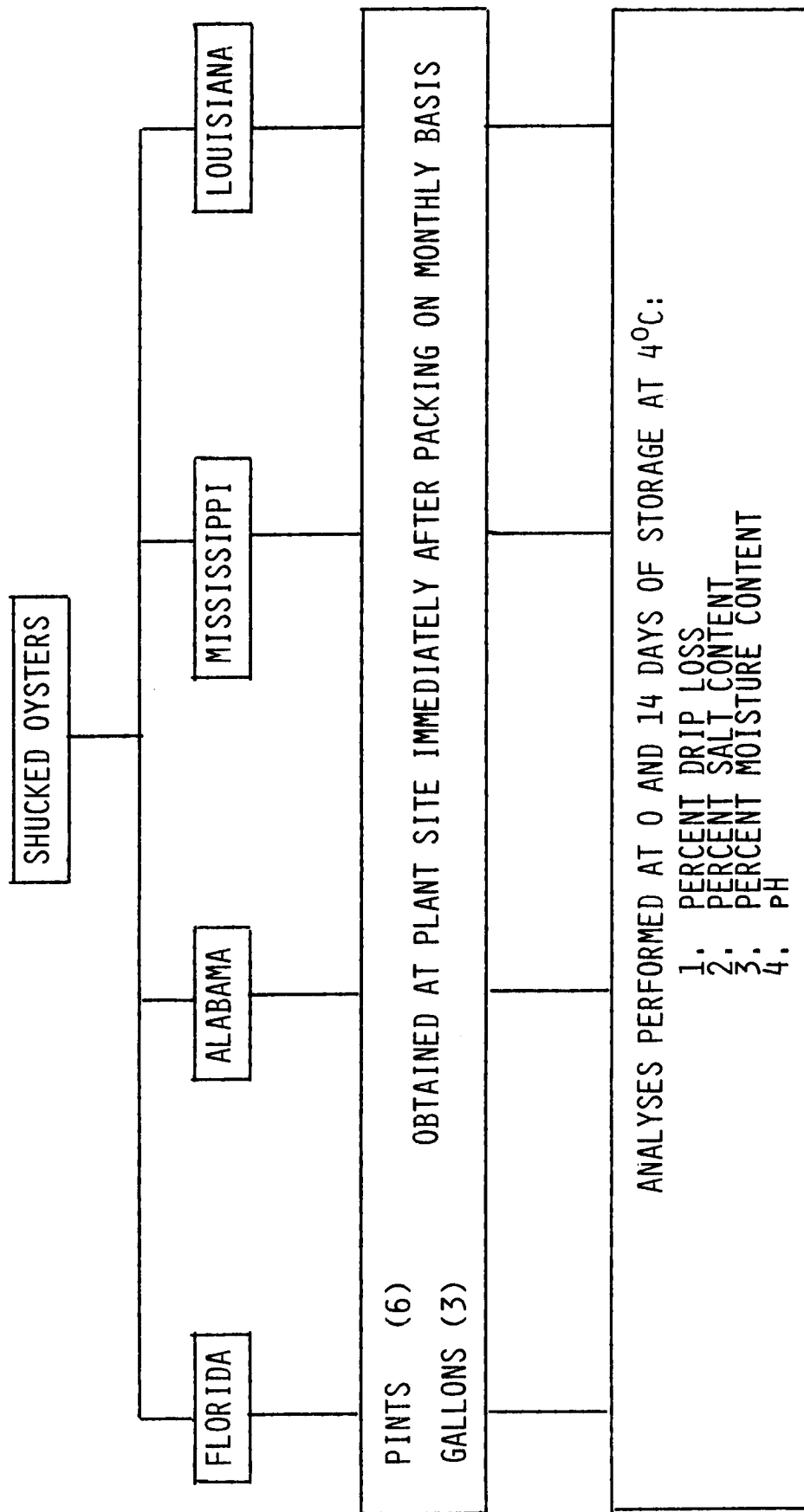
FIGURE 1. FLOW SHEET DESCRIBING THE PLAN OF WORK FOR OBTAINING DRIP LOSS DATA ON COMMERCIALLY PRODUCED SHUCKED OYSTERS FROM STATES BORDERING THE GULF OF MEXICO.

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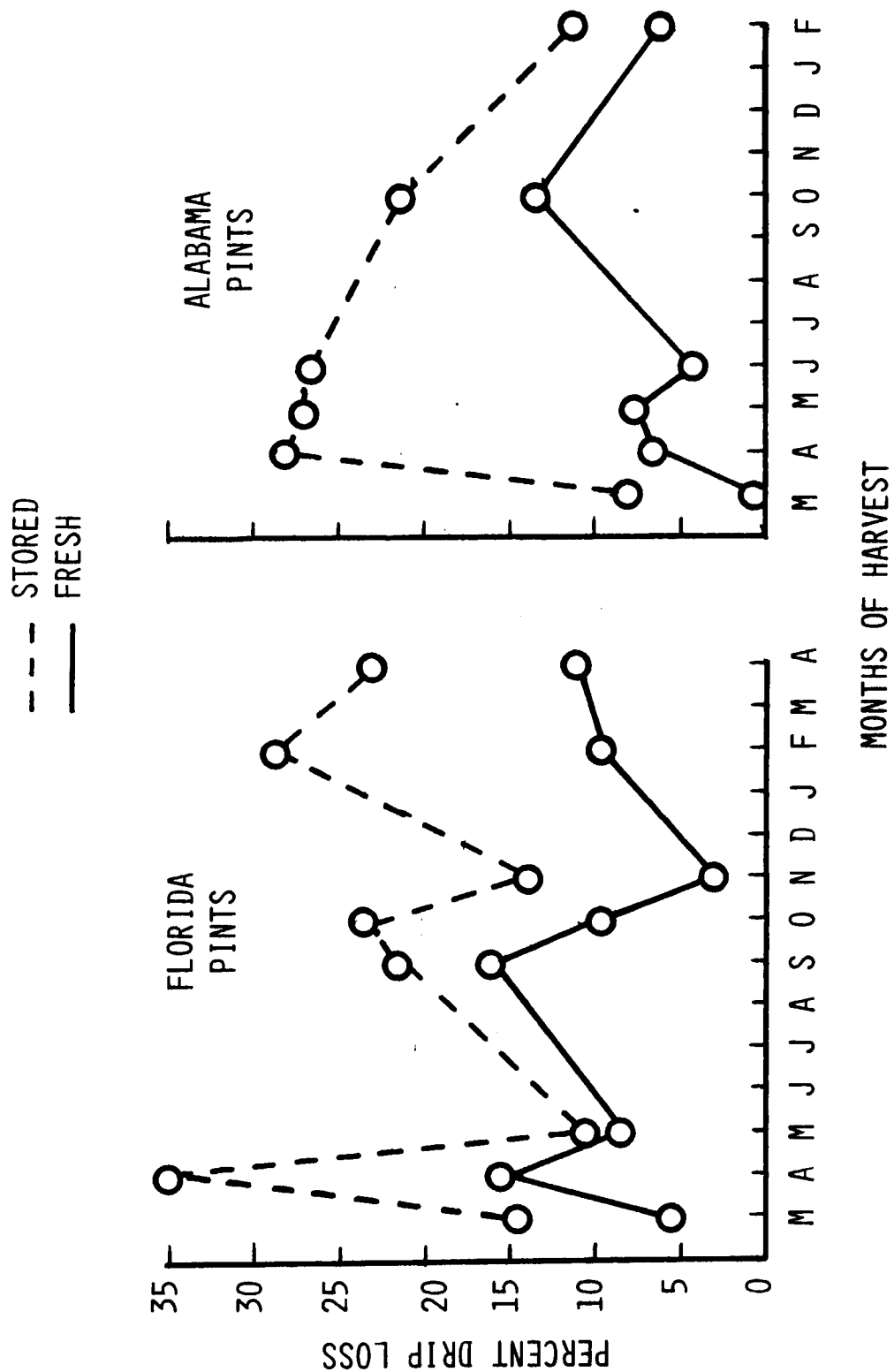
FIGURE 2. PERCENT DRIP LOSS FROM PINTS OF FRESH AND STORED OYSTERS OF COMMERCIAL STOCK FROM FLORIDA AND ALABAMA DURING ONE YEAR OF PRODUCTION.
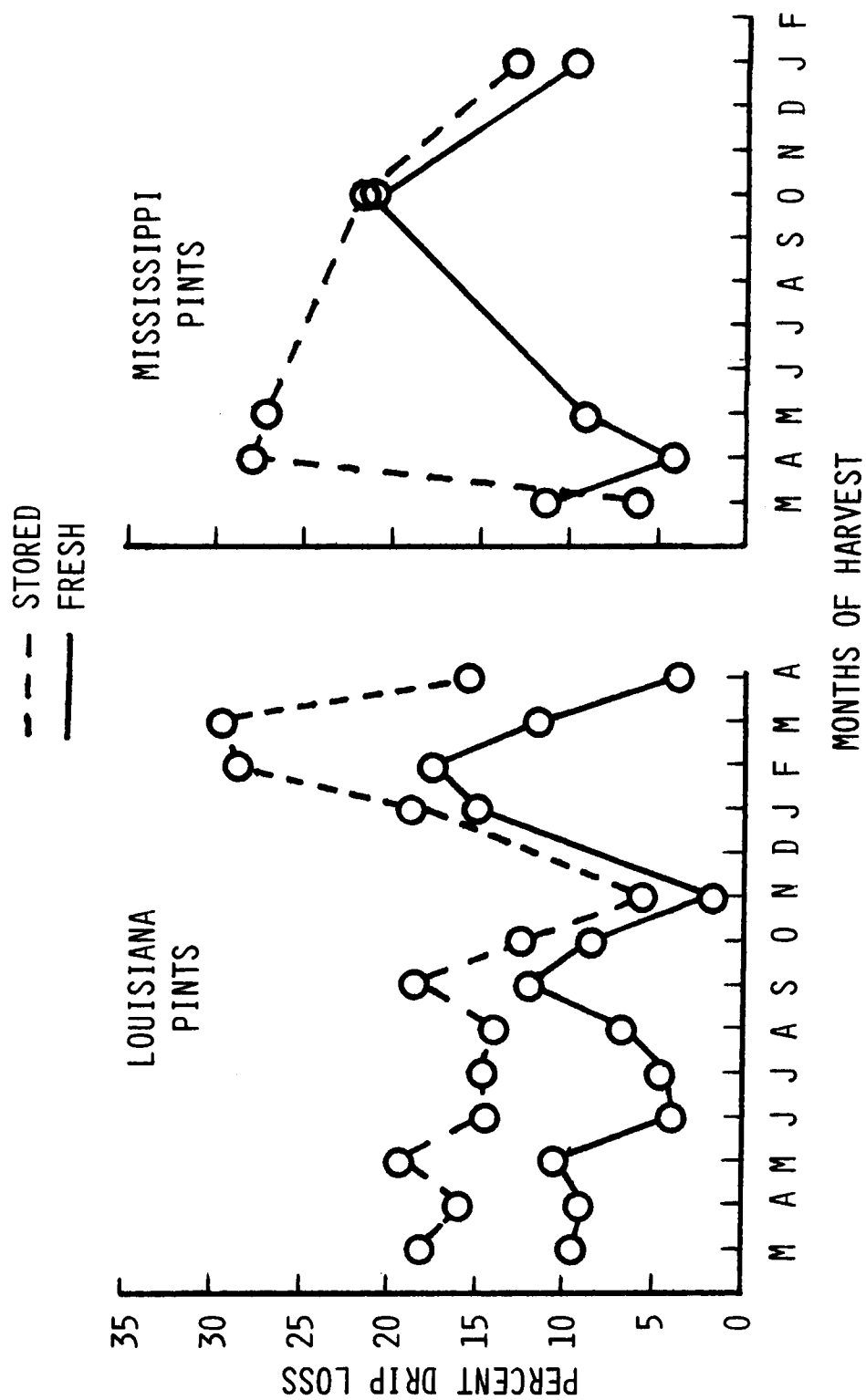
FIGURE 3. PERCENT DRIP LOSS FROM PINTS OF FRESH AND STORED OYSTERS OF COMMERCIAL STOCK FROM LOUISIANA AND MISSISSIPPI DURING ONE YEAR OF PRODUCTION.
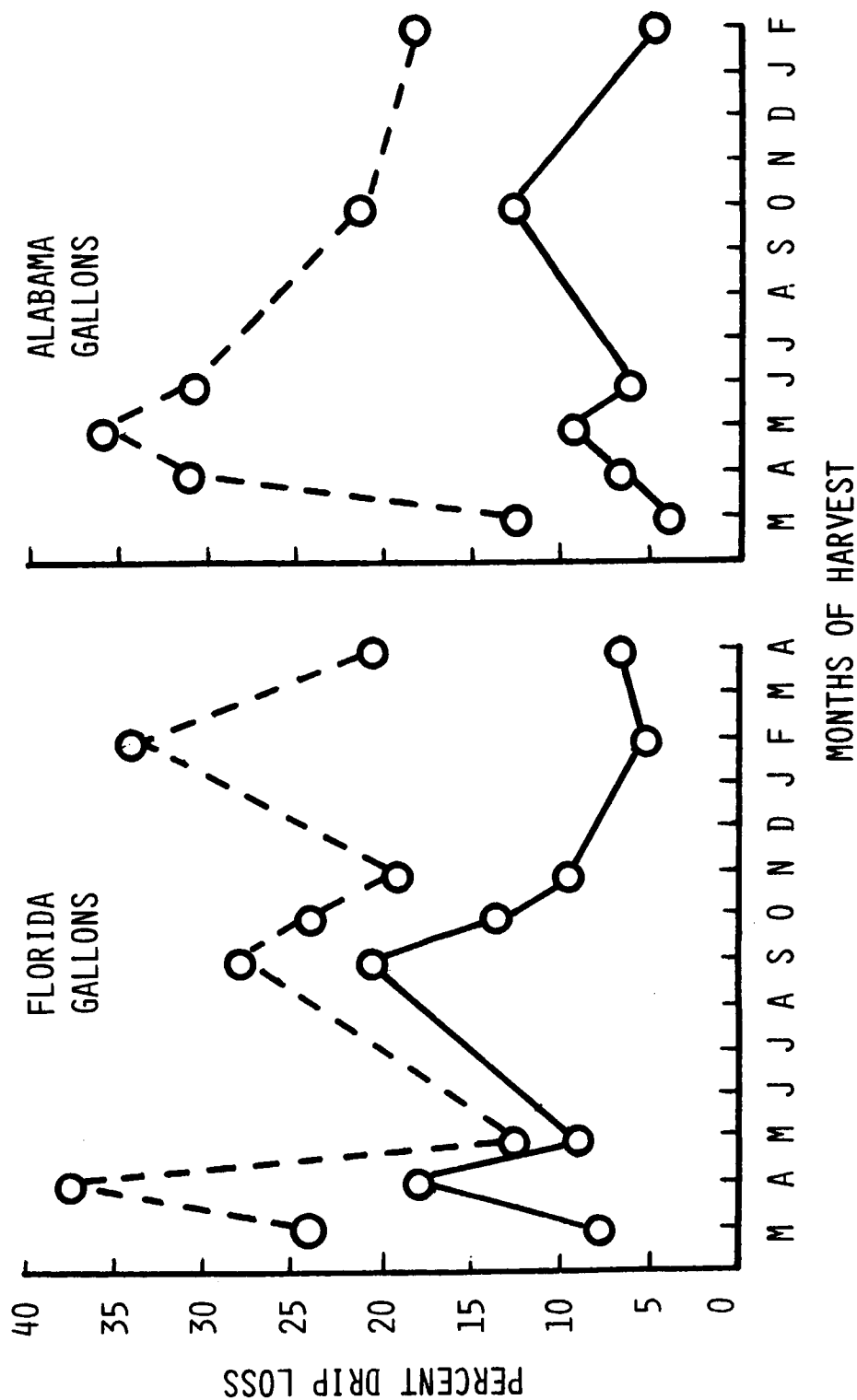
FIGURE 4. PERCENT DRIP LOSS FROM GALLONS OF FRESH AND STORED OYSTERS OF COMMERCIAL STOCK FROM FLORIDA AND ALABAMA DURING ONE YEAR OF PRODUCTION.

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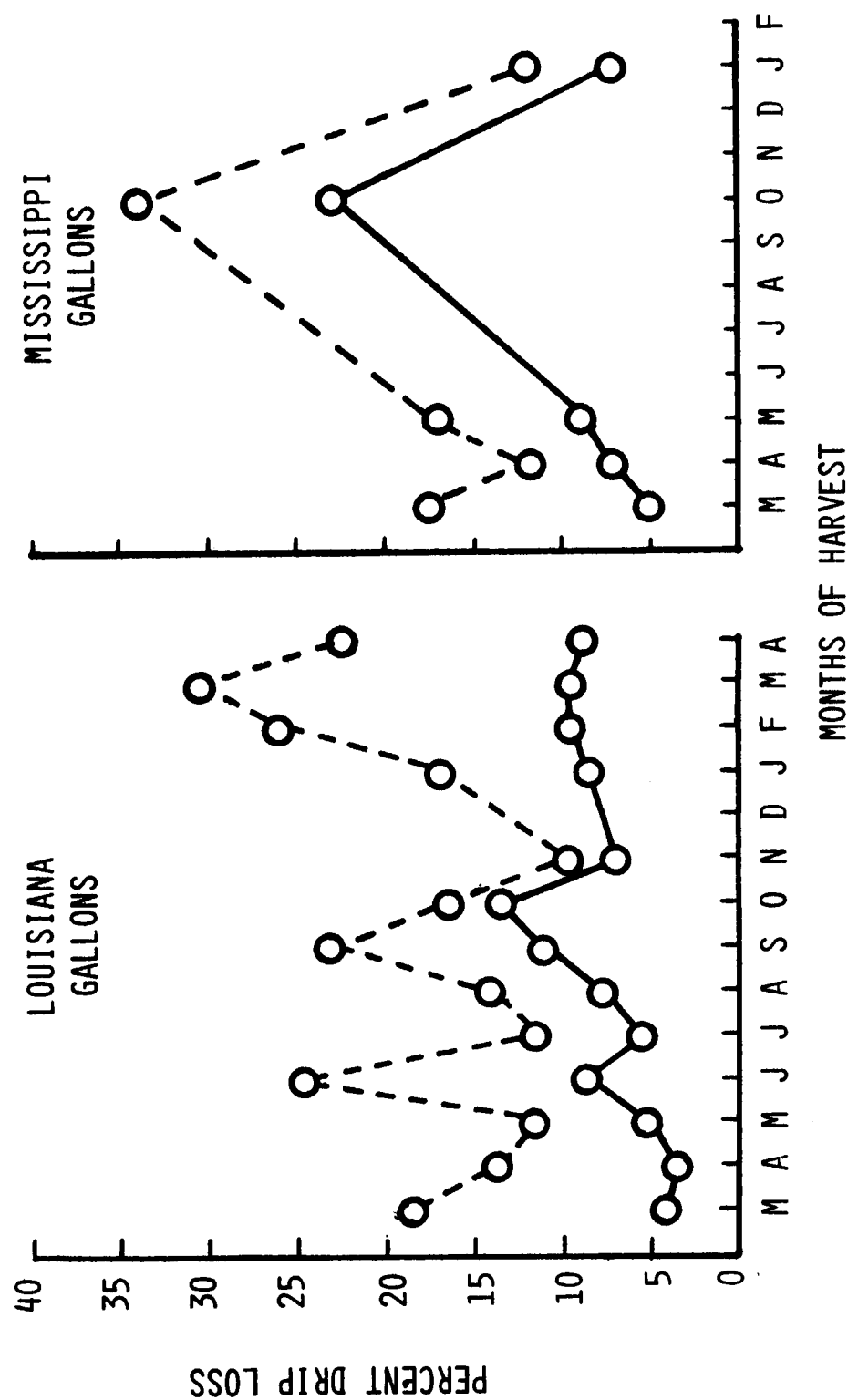
FIGURE 5. PERCENT DRIP LOSS FROM GALLONS OF FRESH AND STORED OYSTERS OF COMMERCIAL STOCK FROM LOUISIANA AND MISSISSIPPI DURING ONE YEAR OF PRODUCTION.

BIOECONOMIC ASSESSMENT OF A
POULTRY AND TILAPIA AQUACULTURE SYSTEM

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INTRODUCTION

Considerable interest has been shown by both academic and commercial concerns over the rapidly developing field of aquaculture. Realization of the limited nature and variability of supply from wild stocks of aquatic organisms, coupled with rapidly increasing biological and technical knowledge have led to a worldwide interest in the development of aquatic husbandry. One method used to enhance fish production is the utilization of animal wastes (5). Such wastes, when discharged into ponds, supply nutrients that enhance phytoplankton growth. The phytoplankton may be utilized as a food source by certain fish species, notably tilapia.

Poultry production systems involving chickens, create waste disposal problems when large numbers of birds are kept in close proximity. Each adult bird (4 lbs.) produces a quarter of a pound of fecal material per day (9) creating added costs and problems of disposal. A liquid manure flush waste disposal system for 10,000 birds may produce over one million gallons of waste water annually. There does not seem to be any one best method of handling poultry wastes; each producer must consider his own position and alternatives (9). A producer may choose to collect and air dry the manure. The manure may then be applied to agricultural land or sold as fertilizer. As an alternative, the producer may collect liquid manure from concrete collecting pits. The liquid manure slurry is then applied to agricultural land.

Tilapia aurea are especially suited for use in sewage/aquaculture facilities, since once past fingerling size (25 mm), they feed directly on phytoplankton (4, 10). This ability to utilize the phytoplankton eliminates the need for feeding costly animal protein feeds, as is done in trout and catfish culture. The incorporation of tilapia into ponds whose primary purpose in sewage treatment may actually improve the water quality (11). The entire fish culture/sewage-treatment system can therefore be considered an integrated one, with fish improving the treatment of sewage waste and the sewage increasing the yield of fish within certain limits (8).

Tilapia have potential as a component of livestock feed, fish meal or directly as human food, although there is no developed market for tilapia in the United States. The requirements of the fish

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meal industry best fit the characteristics of tilapia and may represent the most likely early market. Fish meal plants along the Gulf of Mexico process primarily menhaden. Menhaden fishing declines in October and the plants are forced to shut down. Tilapia being harvested in October could extend the production cycle of fish meal plants. The high fat content of tilapia (40-60%) is economically important since fish oil is currently valued at $450 per ton. Depending on the oil content, tilapia could be competitive with menhaden at a price of $.03 to $.04 per pound delivered. For Texas, the closest menhaden fish meal plants are in New Orleans, Louisiana. Truck transportation costs for whole fish over a distance of 500 miles are 2¢ per pound. Therefore, under present market situations tilapia must be raised for less than 2¢ per pound. For human consumption, tilapia may bring $.60 per pound or more. Catfish are currently valued from $.53 to $.70 per pound live weight (14).

This study examines the economic relationships in a commercial poultry egg production facility with a liquid manure handling system that provides for the growth and harvesting of tilapia. The actual structure of the poultry sewage/aquaculture facility is hypothetical since a commercial facility does not exist at present. Regardless, the major individual components of a proposed system are well understood from current research and the important interface of poultry sewage and tilapia growth is currently under intensive study.

MATERIAL AND METHODS

The hypothetical sewage/aquaculture system can be divided into three components: i) tilapia, ii) pond, and iii) poultry. The components of each system are discussed below.

Tilapia Component

The biological system is based on an annual five month (June-October) production cycle and seven idle or overwintering months. During the overwintering period only brood stock are maintained. The techniques and processes of the overwintering cycle are presented first followed by a description of the actual production cycle.

A breeding population of tilapia was maintained year round. This population, of approximately 100 individuals, was kept in a 4 foot deep, 10 foot diameter aluminum tank inside the poultry house or other heated structure. A 1.5 horsepower electric motor (operating cost $.009/hr.) continuously pumped water through the tanks to a 55 gallon biofilter. During the overwintering period brood fish were assumed to be fed a commercial fish food (@ 20¢/lb.) several times a week, a total of 50 pounds. In late May, the brood fish were then transferred to the quarter acre brood pond.

Tilapia will reproduce naturally in the brood ponds within one week to ten days of stocking. The fry will school together near the surface and are easily netted. Fingerlings weigh about 12 gms/100 upon transfer to the production pond (2, 13). The fingerlings were assumed to be treated with 17-alphaethynyltestosterone in the feed in order to insure monosex culture (6). Monosex culture limits uncontrolled breeding during the summer and thereby contributes to a more uniform harvest size. Once the fingerlings are stocked in the main production pond, they receive almost no attention. The owner

checks the ponds weekly for signs of stress, i.e., fish gasping for air. Further, water quality, parameters of dissolved oxygen, ammonium, and temperature are checked with a chemical kit ($200.00 purchase cost). Sewage is pumped continuously into the ponds from holding pits at rates ranging from zero to the entire manure slurry production per month if over six acres of pond area is built.

For this study seven varying rates of pumping result in the levels of manure slurry concentration in the production pond. The concentration levels are: i) zero gallons/acre; ii) 857 gallons/acre; iii) 1,714 gallons/acre; iv) 3,428 gallons/acre; v) 5,142 gallons per acre; vi) 6,857 gallons/acre; and vii) 8,571 gallons/acre. Levels i-iv represent concentrations that have been attempted in present research. Levels v-vii, and the growth of tilapia in ponds receiving these levels is hypothetical. At the end of the growing season, tilapia are harvested by netting or draining the pond and collection of the fish through the drainage pipe.

Two to four men control the harvest depending on pond size. Pond discharge may be disposed of on agricultural land with 20 acres needed to dispose of one acre-foot of water. If relatively flat land is available, a gravity drainage ditch system may be used. In hilly regions a mechanical irrigation system must be used. A sprinkler irrigation system is estimated at $125 per acre per year.

Poultry Component

The components of the poultry system are based on a series of assumptions on the type of operation that is to be considered. The component of the poultry system that is of primary interest involves poultry manure waste handling. The poultry unit is assumed to be located in a southern part of the United States allowing a five month (June-October) production period for tilapia. The poultry house contains 10,000 adult laying hens (4 lbs.) which are kept in an enclosed cage confinement system. Open wire mesh cages allow manure to collect in a concrete pit located in the floor of the poultry house. Water is added to obtain a slurry that is approximately 15% solids. The sewage volume, including additional water, amounts to 1,406 gallons per 10,000 chickens per day (3). The manure slurry is removed every 60 days via a 1000 gallon vacuum system commonly called a "honey wagon". The tank is transported by tractor to agricultural land and is applied to the surface by releasing the slurry while the tank is in motion.

For each individual poultry producer, either positive or negative benefits may be accrued from the sewage handling process, depending on the producer's ability to effectively utilize the poultry manure. The primary positive benefit is in the nutrient value and its usefulness in agricultural production. The value of the manure is in the money saved by not utilizing commercially purchased fertilizers. Taking an average nutrient analysis of 1000 gallons of manure slurry (85% moisture, 64 lbs. nitrogen, 28 lbs. phosphate, 59 lbs. potash) (1), the present market price was determined for the equivalent commercially available fertilizer. The price for N is 21¢/lb., for P, 14¢/lb. and for K, 14¢/lb. This results in a maximum value of $25.62 for 1000 gallons of manure slurry, with progressively lower values being assigned manure that is not effectively due to land or labor restrictions or contains less nutrients.

The negative benefits of poultry manure are economic and aesthetic. The total cost of collecting, transporting and applying liquid manure has been estimated for three operations utilizing surface spread or subsoil injection. Values for disposal costs averaged $3.00/1000 gallons. The costs include a fixed cost on the tractor operation and labor (12). Another "cost" of field disposal is the odor and fly problems that may occur if subsoil injection is not used. Also, there is the possibility of groundwater contamination. The importance of each factor will vary among producers. Costs that should be considered in the production decisions involving how to dispose of manure are the manure disposal costs that remain variable. This study assumed the poultry operator already owns a tractor, storage tank and honey wagon, and that the interest and depreciation associated with the items are fixed costs. Of the total costs of $3.00/1000 gallons, variable costs are estimated to be $1.25/1000 gallons.

Pond Component

The pond system, consisting of pond characteristics and construction specifications, was developed from data obtained from catfish producers (6). In this study it was assumed that the poultry operator builds a single production pond, varying in size from one to ten surface acres. The range of sizes allows a choice of pond sizes within a reasonable range for a producer with 10,000 hens. Larger pond sizes allow for a greater total number of tilapia to be stocked and harvested. Also larger pond sizes utilize a greater total amount of manure slurry. Additionally, a 0.1 acre brood pond was built with one common side with the production pond. The ponds were built with bulldozers on a level site. Two levees (one length and one width) had 12 foot crowns and 42 foot bases while the other two and the brood pond had six foot crowns and 36 foot bases. The pond shape was rectangular, with the longer dimension being approximately three times the width. The depth of the ponds averaged four feet with the bottom having a slight slope (0.2'/100') end to end to facilitate drainage. The drainage system of each pond was constructed of various sizes of steel drainage pipe. An electric pump was supplied for the production and brood ponds and would maintain at least 50 gpm per surface acre.

The method of analysis utilized for the poultry sewage/tilapia culture facility was linear programming. The hypothetical situation assumed that pond construction decisions are to be determined and that no ponds would initially exist. Linear programming maximizes a series of linear equations representing the inputs used by each activity. In the analysis the poultry operator has four basic decisions to make: i) what size pond to build; ii) what month or months in which to produce tilapia; iii) what amount of manure slurry to pump to the ponds; and iv) how to dispose of manure not utilized in the ponds.

The optimal decision was based on maximum annual net returns to the operator. The optimal decision was made for three different poultry systems. Each system was characterized by a unique value of the manure when it is applied to agricultural land. The values chosen represent the lowest expected benefits that a poultry operator could face, and were expected to supply the lowest price of tilapia necessary to initiate tilapia production. Gross value of the manure is the value of the nutrients contained in the manure. Net value of the manure is the value of the manure after accounting for a $1.25/1000

gallon cost of application. The net values of the manure slurry were: -1.25/1000 gallons, $0.0/1000 gallons, and $1.60/1000 gallons. In the analysis, the price of tilapia was varied from $0.0 to $.60 live weight.

RESULTS AND DISCUSSION

The results of the linear programming analysis of the poultry operations intiial decision on whether or not to initiate tilapia production are presented in Tables 1-6. The three main farm situations used were: i) manure slurry has a $0.0/1000 gallon gross value (Tables 1 and 2); ii) manure slurry has a $1.25/1000 gallon gross value (Tables 3 and 4); and iii) manure slurry has a $2.85/1000 gallon gross value (Tables 5 and 6). Tables 1,3, and 5 present the results for operator's utilizing a stocking density of 3000 tilapia per acre whereas Tables 2, 4, and 6 are stocking density of 5000 tilapia per acre, a hypothetical stocking density.

In Table 1, the potential annual net benefit (NB) obtained from both tilapia production and manure slurry disposal is presented for five pond sizes. Disposal may take place either in the summer or winter. If no pond is built (pond size 0) then all of the manure must be disposed of on agricultural fields. In the summer 211,800 gallons (col. 2) and in the winter 296,500 gallons (col. 3). With no pond built and zero value for the slurry, the total cost of disposal of the manure is $635 (col. 7). With a zero value for the slurry, cost can be reduced by utilizing a one acre pond both summer (col. 4) and winter (col. 5) at a cost of $472 (col. 7). An alternative production strategy exists for the one acre pond that results in the same annual net benefit. Tilapia production utilizes manure during the summer months at a rate depending on the concentration and pond size. For the one acre pond 25,700 gallons (col. 4) of manure slurry are supplied the ponds over a five month period. Since the tilapia are being cultured over the summer months excess manure slurry not utilized by the tilapia may not be disposed of through the ponds. Therefore annual field disposal of manure slurry amounts to 186,100 gallons (col. 2). Annual pond disposal of manure in the winter remains at 296,500 gallons (col. 5). Annual costs are divided between manure slurry disposal and tilapia production. Total manure slurry disposal costs amount to $281 (col. 7). If the producer receives $.10 per pound (live weight) for the tilapia gross receipts (GR) from tilapia production amount to $254 (col. 10). Total costs, (TC), including both fixed and variable costs of owning a pond and operating a tilapia production system, amount to $445 annually (col. 11). The resulting net income (NI), derived from GR and TC (GR - TC = NI) amounts to -$192 (col. 12), an annual loss. Adding the NI of manure slurry disposal -$281, and the NI from tilapia production -$191.85 results in an annual NI of $472 (col. 14). The price of tilapia necessary to result in an annual cost that is equal to that of pond disposal of manure is identified as the breakeven dollars per pound ($101) (col. 13). At this price, tilapia production in a one acre pond in combination with manure slurry disposal will result in an annual benefit equal to pond disposal of all manure.

In a two acre pond tilapia production utilizes a total of 51,420 gallons of manure over a five month period, with the remainder of the summer's production of manure, 160,400 gallons, being disposed

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Pond Size ac. (1)	Annual Manure Slurry Disposal				Costs and Returns From Manure Slurry			Tilapia Production				Breakeven Price $/lb. (13)	Annual Net Income From Slurry And Tilapia (14)
	Field		Pond		Gross Receipts (6)	Total Costs (7)	Net Income (8)	Pounds (9)	Gross Receipts (10)	Total Costs (11)	Net Income (12)		
	Summer (2)	Winter (3)	Summer (4)	Winter (5)									
	----1000 gal.----				----$----				----$----				$
0	211.8	296.5	--	--	--	635	-635	--	--	--	--	--	-635
1	--	--	211.8	296.5	--	472	-472	--	--	--	--	--	-472
1	186.1	--	25.7	296.5	--	281	-281	2,513	254	445	-192	.101	-472
2	160.4	--	51.4	296.5	--	249	-249	5,026	503	643	-140	.083	-389
3	134.7	--	77.1	296.5	--	217	-217	7,539	754	823	-69	.075	-286
4	109.0	--	102.8	296.5	--	185	-185	10,053	1,005	1,054	-49	.076	-234
5	83.3	--	128.6	296.5	--	153	-153	12,566	1,257	1,211	46	.070	-107

Table 1. Annual Production Strategies and Resulting Profit/Loss for Three Poultry Sewage Disposal Methods: i) Field Disposal ii) Pond Disposal iii) Tilapia Production, by Pond Size, where the Manure is Valued at $0 per 1000 Gallons, Stocking Density is 3000/Acre, and $.10 per pound for tilapia.

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of onto the fields. Winter pond disposal amounts to 296,500 gallons. The total cost of slurry disposal is $249. Tilapia production results in 5,026 pounds with GR equaling $503, holding the price constant at $.20 per pound. TC amounts to $642.57 annually. The resulting NI equals a loss of $140 for tilapia production. Combining the costs of manure slurry disposal ($249) and the NI from tilapia production results in a combined loss of $389 annually. If the price of tilapia was equal to the breakeven price of $.083 per pound the resulting combined annual net benefit from slurry and tilapia will be $472, an amount equal to pond disposal of all manure.

For a three acre pond, tilapia production utilized 77,100 gallons of manure over the five month production period. The remainder of the summer production of manure slurry, 134,700 gallons, was disposed of on fields. TC slurry disposal amounts to $217. GR for tilapia equals $754 ($.10 per pound) and TC equals $823 with a resulting NI of -$69. Combining the NI of manure slurry -$217 and NI of tilapia production -$69 results in an annual combined net income of -$286. If the price of tilapia is at the breakeven of $.076 per pound then the resulting combined annual net benefit would be -$472.

When a four acre pond is built, the resulting production of tilapia is 10,053 pounds utilizing 102,800 gallons. Slurry disposal TC is $185. Tilapia production results in NI of -$49. Combining the NI from manure slurry and tilapia production results in a combined annual net benefit of -$234. If the price of tilapia were $.076 per pound the resulting combined annual net benefit is -$472.

For a five acre pond, the largest size considered for the hypothetical poultry system the tilapia production amounted to 12,566 pounds with a resulting GR of $1,257. Total manure use in tilapia productions was 128,550 gallons, with the remainder (83,300 gallons) going to field disposal. Manure disposal TC were $153. The total cost of tilapia production was $1,211 annually with a resulting NI of $46. The combined annual net benefit remains a loss of $107 annually. If the price of tilapia was at the breakeven price of $.070 per pound the resulting combined net benefit would be $472 loss annually.

In Table 2 stocking density is 5000 instead of 3000 per acre and the price received per pound of tilapia is $.06 instead of $.10. The pounds of tilapia harvested ranged from 4,180 pounds for one acre to 20,900 pounds for five acres. For a one acre pond production system the GR from tilapia production is $225. With total costs of $446, the NI amounts to a loss of $192. The combined net benefit from manure slurry and tilapia production was a loss. This compares with results in Table 1 where pounds harvested ranged from 2,513 to 12,566 for the one and five acre ponds respectively. This assumes that the greater stocking density had no effect on the growth of the fish at the levels considered. The price received for tilapia is reduced from $.10 to $.06 and the entire facility receives the same annual net benefit from slurry and tilapia (col. 14) for all pond sizes. The breakeven price is reduced at the higher stocking density. For example, a five acre pond must receive $.043 per pound at 5000 stocking density whereas with 3000 stocking density the price was over $.070.

In Table 3 and Table 4 the costs and returns are presented for a poultry operator who is able to recover $1.25 per thougand gallons worth of the nutrient value of the manure slurry. The net costs of field disposal of manure slurry is therefore $0.0/1000 gallons.

Pond Size ac.	Annual Manure Slurry Disposal				Costs and Returns From Manure Slurry			Tilapia Production					Annual Net Income From Slurry And Tilapia
	Field		Pond										
	Summer	Winter	Summer	Winter	Gross Receipts	Total Costs	Net Income	Pounds	Gross Receipts	Total Costs	Net Income	Breakeven Price	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	$/lb. (13)	$ (14)
	----1000 gal.----				----$----				----$----				
0	211.8	296.5	--	--	--	635	-635	--	--	--	--	--	-635
1	--	--	211.8	296.5	--	472	-472	--	--	--	--	--	-472
1	186.1	--	25.7	296.5	--	281	-281	4,180	255	446	-192	.061	-472
2	160.4	--	51.4	296.5	--	249	-249	8,360	510	649	-140	.051	-389
3	134.7	--	77.1	296.5	--	217	-217	12,540	765	834	-69	.047	-286
4	101.0	--	102.8	296.5	--	185	-185	16,720	1,020	1,060	-49	.047	-234
5	83.3	--	152.7	296.5	--	153	-153	20,900	1,275	1,231	46	.043	-107

Table 2. Annual Production Strategies and Resulting Profit/Loss for Three Poultry Sewage Disposal Methods: i) Field Disposal ii) Pond Disposal iii) Tilapia Production, by Pond Size, where the Manure is Valued at $0 per 1000 Gallons, Stocking Density is 5000/Acre, and $.06 per pound for tilapia.

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Table 3. Annual Production Strategies and Resulting Profit/Loss for Three Poultry Sewage Disposal Methods: i) Field Disposal ii) Pond Disposal iii) Tilapia Production, by Pond Size, where the Manure is Valued at $1.25 per 1000 Gallons, Stocking Density is 3000/Acre, and $.18 per pound for tilapia.

Pond Size ac.	Annual Manure Slurry Disposal				Costs and Returns From Manure Slurry			Tilapia Production				Breakeven Price	Annual Net Income From Slurry And Tilapia
	Field		Pond										
	Summer	Winter	Summer	Winter	Gross Receipts	Total Costs	Net Income	Pounds	Gross Receipts	Total Costs	Net Income		
	-----1000 gal.-----				-------$-------				-------$-------			$/lb.	$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
0	211.82	296.5	--	--	635	635	0	--	--	--	0	--	0
1	186.1	296.5	25.7	--	604	604	0	2,513	445	445	0	.1	0
2	160.4	296.5	51.4	--	571	571	0	5,026	891	643	248	.13	248
3	134.7	296.5	77.1	--	539	539	0	7,539	1,336	823	513	.11	513
4	109.0	296.5	102.8	--	507	507	0	10,053	1,782	1,054	727	.10	727
5	83.3	296.5	128.6	--	475	475	0	12,565	2,227	1,210	1,010	.0?	1,010

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Pond Size ac. (1)	Annual Manure Slurry Disposal				Costs and Returns From Manure Slurry			Tilapia Production				Breakeven Price $/lb. (13)	Annual Net Income From Slurry And Tilapia $ (14)
	Field		Pond		Gross Receipts (6)	Total Costs (7)	Net Income (8)	Pounds (9)	Gross Receipts (10)	Total Costs (11)	Net Income (12)		
	Summer (2)	Winter (3)	Summer (4)	Winter (5)									
	----1000 gal.----				----$----				----$----				
0	211.8	296.5	--	--	635	635	0	--	--	--	--	--	0
1	186.1	296.5	25.7	--	604	604	0	4,180	445	445	0	.106	0
2	160.4	296.5	51.4	--	571	571	0	8,360	890	649	241	.07?	241
3	134.7	296.5	77.1	--	539	539	0	12,540	1,336	833	502	.066	502
4	109.0	296.5	102.8	--	507	507	0	16,720	1,780	1,068	713	.063	713
5	83.3	296.5	128.6	--	475	475	0	20,400	2,225	1,230	996	.05?	996

Table 4. Annual Production Strategies and Resulting Profit/Loss for Three Poultry Sewage Disposal Methods: i) Field Disposal ii) Pond Disposal iii) Tilapia Production, by Pond Size, where the Manure is Valued at $1.25 per 1000 Gallons, Stocking Density 5000/Acre, and $.106 per pound for tilapia.

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Tables 3 and 4 present the results for a stocking density of 3000 and 5000 tilapia per acre, respectively.

When the poultry operator is able to obtain a benefit at $1.25 per 1000 gallons of manure slurry, the costs of application ($1.25 per 1000 gallons) are covered. When no pond is built, the operator disposes of the entire manure slurry amount via annual field disposal at a TC of $635 annually, resulting in annual NB of $0.

Tilapia production is initiated in a one acre pond at $.18 and $.106 per pound of tilapia in Tables 3 and 4 respectively. This relationship is expected since the higher stocking density produces more total pounds of fish such that a lower breakeven price is required to initiate production. It is important to note that the breakeven prices in Tables 3 and 4 are greater than Tables 1 and 2. In addition, the higher value of manure on agricultural fields and the higher price received for tilapia results in the annual NB from the manure slurry and tilapia production being positive. The annual net benefit ranged from $0 for one acre of tilapia production to $1,011 and $996 for five acres at 3000 and 5000 stocking per acre respectively (Tables 3 and 4, respectively, col. 1).

Tables 5 and 6 present the results of costs and returns for the poultry operator who is able to realize $2.85 per 1000 gallons benefit from the manure slurry. The stocking density is 3000 per acre for Table 5 and 5000 per acre for Table 6. The important difference in Tables 5 and 6 is the decreased concentration of manure slurry going into the ponds for tilapia production.

The GR from manure slurry increased over previous levels due to the increased amount of manure slurry applied to the fields as well as the increased price benefit of slurry. The net income from slurry ranged from $786 for a one acre pond to $676 for slurry disposal in conjunction with a five acre pond (Tables 5 and 6). Due to the lower concentration of manure the pounds of tilapia produced were lower for each pond size. Production for a one acre pond was 2,480 pounds, up to 12,401 pounds for a five acre pond at a stocking density of 3000/acre (Table 5). At a price of $.23 per pound the GR from tilapia was $580 for a one acre pond, $1,711 for a three acre and $2,852 for a five acre. The net income tilapia increased from $110 for one acre to $1,623 for five acres. The combined annual NB rose to $2,300 annually for a five acre system. The breakeven price was lowest for a five acre system, at $.11 per pound of tilapia which was $.04 greater than the system in Table 1.

Summary

The utilization of animal waste in aquaculture fish production efficiently disposes of animal waste. Tilapia are especially suited for use in a sewage/aquaculture facility, since they feed directly on phytoplankton. Tilapia has potential as a component of livestock feed, fish meal or directly as human food, although there is no developed tilapia market in the U.S. at this time.

This study examined the economic relationships in a commercial poultry egg production facility with a liquid manure handling system that provides for the growth and harvest of marketable fish. The poultry house contains 10,000 laying hens and was assumed to be located in the southern part of the United States which allowed a five month growing period for tilapia. The manure could be used on agricultural

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Table 5. Annual Production Strategies and Resulting Profit/Loss for Three Poultry Sewage Disposal Methods: i) Field Disposal ii) Pond Disposal iii) Tilapia Production, by Pond Size, where the Manure is Valued at $2.85 per 1000 Gallons, and Stocking Density 3000/Acre, and $.23 per pound for tilapia.

Pond Size ac. (1)	Annual Manure Slurry Disposal				Costs and Returns From Manure Slurry			Tilapia Production				Breakeven Price $/lb. (13)	Annual Net Income From Slurry And Tilapia (14)
	Field		Pond		Gross Receipts (6)	Total Costs (7)	Net Income (8)	Pounds (9)	Gross Receipts (10)	Total Costs (11)	Net Income (12)		
	Summer (2)	Winter (3)	Summer (4)	Winter (5)									
	—1000 gal.—				—$—				—$—				
0	211.8	296.5	--	--	1,449	635	813	--	--	--	--	--	813
1	194.7	296.5	17.1	--	1,400	614	786	2,480	580	469	110	.200	896
2	177.5	296.5	34.3	--	1,351	593	758	4,960	1,16.	660	48?	.144	1,239
3	160.4	296.5	51.4	--	1,302	571	731	7,441	1,711	841	87.	.124	1,601
4	143.3	296.5	68.6	--	1,253	550	704	9,920	2,?6?	1,072	1,2?.	.119	1,913
5	126.1	296.1	85.7	--	1,204	528	676	12,401	2,632	1,229	1,6?1	.110	2,???

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Pond Size ac.	Annual Manure Slurry Disposal				Costs and Returns From Manure Slurry			Tilapia Production				Breakeven Price $/lb.	Annual Net Income From Slurry And Tilapia $
	Field		Pond		Gross Receipts	Total Costs	Net Income	Pounds	Gross Receipts	Total Costs	Net Income		
	Summer	Winter	Summer	Winter									
	--1000 gal--				----$----				---$---				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
0	211.8	296.5	--	--	1,449	635	813	--	--	--	--	--	813
1	191.7	296.5	13.1	--	1,400	614	786	4,125	578	457	117	.119	896
2	177.5	296.5	34.3	--	1,351	592	758	8,250	1,155	674	431	.088	1,239
3	160.4	296.5	51.42	--	1,302	571	731	12,375	1,733	862	870	.076	1,601
4	143.3	296.5	68.6	--	550	704	704	16,500	2,310	1,101	1,209	.073	1,713
5	126.1	296.5	85.7	--	1,204	528	676	20,625	908	1,264	1,623	.067	2,300

Table 6. Annual Production Strategies and Resulting Profit/Loss for Three Poultry Sewage Disposal Methods: i) Field Disposal ii) Pond Disposal iii) Tilapia Production, by Pond Size, where the Manure is Valued at $2.85 per 1000 Gallons, and Stocking Density 5000/Acre, and $14 per pound for tilapia.

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fields, disposed of in pond lagoons or used in the pond to produce tilapia. Depending on each producers' situation the net value of a 1000 gallon of manure was assumed to be -$1.25/1000 gallons (equal to the cost of disposing of manure on agricultural land with no value of manure to farmer), $0.00/1000 gallons (value of manure on agricultural land just offsets costs), and $2.85/1000 gallons (value of manure on agricultural land exceeds the costs of disposal). Finally, two stocking densities of tilapia, 3000 and 5000 per acre were considered. Linear programming maximized annual net returns to determine: i) what size pond to build; ii) what months in which to produce; iii) what amounts of manure slurry should be pumped into the ponds; and iv) how to dispose of manure not utilized in the ponds.

For the poultry operator who is unable to realize any of the nutrient benefits of manure slurry on agricultural land the one acre lagoon system is a less expensive way to dispose of manure than spending it on agricultural land. However, the maximum level of biological production was the optimal choice in terms of least costs annually. At a price of $.10 per pound and a stocking density of 3000/acre, all pond sizes produced lower costs than sole operation of a lagoon system. At a breakeven price of $.07 per pound, a five acre production system producing 12,566 pounds of tilapia resulted in costs equal to a lagoon system. Increasing stocking density to 5000 per acre lowered the breakeven price to $.043 for a similar five acre production system.

With increased value of poultry manure to $1.25/1000 gallons and a stocking density of 3000/acre, the value of tilapia necessary to initiate production was estimated to be $.18 per pound. For a five acre pond the breakeven price was $.08 per pound with production of 12,566 pounds. An increased stocking density and resulting greater harvest lowered the price that must be obtained for tilapia. At 5000 tilapia per acre the price necessary to initiate production is $.058 per pound given production of 20,400 pounds for five acres.

With increased value of the poultry manure ($2.85 per 1000 gallons), the amount used in tilapia production fell to 17,100 gallons per acre for five months compared to 25,700 per acre (at lower prices for manure), with the remainder of the manure being disposed of on agricultural land. The price of tilapia necessary to initiate production increased with increased value of manure. With a stocking density of 3000 per acre the price of tilapia had to be $.20 per pound for a one acre pond system. To break even with manure slurry disposal a price of $.11 per pound was necessary for a five acre pond producing 12,401 pounds. With an increased stocking density of 5000 per acre the breakeven price fell to $.067 per pound.

CONCLUSIONS

In order to obtain maximum value, the value of tilapia and the value of the manure as fertilizer must compete economically for the input of manure slurry. In order for poultry operators to make an economically sound decision the manure slurry must be diverted to the particular production system or combination of systems that will produce the maximum benefit per unit of manure slurry. As the value of manure slurry increases, the price of tilapia must necessarily be higher before tilapia production will be initiated. An operator who is unable to realize the benefits of manure due to inadequate land or

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labor would be able to initiate production of tilapia at $.045 per pound for a five acre production system, five month growing period, with a stocking density of 5000 tilapia per acre.

The marketing of tilapia has not been extensively researched. Research is underway on utilization of tilapia as a poultry feed component. Utilization of tilapia as a component of fish meal would depend on large volumes of production and costs per pound of 2¢ or less.

At the present early stage of biological research on production of tilapia utilizing poultry manure, the production already achieved should allow serious consideration being given the production of tilapia as an alternative use of manure slurry, provided the proper market for tilapia can be developed.

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MARKET POTENTIAL FOR SARDINES FROM THE GULF OF MEXICO

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Sardines and sardine-like fishes are relatively abundant in the Gulf of Mexico. Houde (1) in a 1975 article reported that the potential maximum sustainable harvest of all sardine-like species in the Eastern Gulf of Mexico would approximate 525,000 metric tons annually.

This paper will deal exclusively with the potential for the development of a Gulf fishery based on the Spanish Sardine (Sardinella anchovia). While no estimates were made specifically for Spanish Sardines, Houde reported that their biomass could be as large as 250,000 metric tons. Fishermen who produce Spanish Sardines for human consumption and for bait indicate that the species is relatively abundant in the areas where they fish.

The Sardinella anchovia is on the Codex Alimentarius as an approved species that may be labeled as sardines. When these standards are accepted the fish products must be labeled as to the geographical area in which they are produced and packed. Therefore, the names "Gulf Sardines" or "Sardines from the Gulf of Mexico" may be considered as possible names.

Houde used a technique proposed by Alverson and Pereyra (1969) and Gulland (1971 and 1972) to make a preliminary estimate of the potential annual yield based on biomass estimates.

$$C_{max} = XMB_0$$

where:

C_{max} = Maximum annual sustainable harvest

X = Percent of initial stock from which MSH can be obtained (0.5)

M = Instantaneous rate of natural mortality

B_0 = Initial stock size (virgin biomass)

Mortality (M) for subtropical and tropical clupeid fish species range from 0.5 to 1.0.

Houde concludes, "If Spanish Sardine biomass is 250,000 m.t., they could contribute from 62,500 to 125,000 m.t. to the annual yield...." (1, p. 80)

Market Potential

There is a potentially large market for canned sardines from the Gulf of Mexico. Total production of sardines in the United States has exhibited wide fluctuations since 1965, varying from a low of 19 million pounds in 1970 to a high of 37 million pounds in 1972. During the most recent three year period, the production has averaged 26 million pounds, as shown in Table 1.

The total dollar value of sardine production in the U. S. has increased steadily from $11 million in 1965 to $25 million currently. The price per pound has also increased and has maintained a level of $0.96 during the past two years.

Imports constitute the majority of sardines sold in this country. Over the period 1965 to the present, imports averaged two-thirds of the total U. S. sardine supply, as shown in Table 2. The average volume imported has been near 60 million pounds annually. Also, the dollar value of imported sardines has been considerably higher than domestically produced sardines since 1965, Table 3. Figure 1 shows the relative contribution of domestic and imported sardines to the total U. S. supply.

Sardines from the Gulf of Mexico could be expected to achieve a market share of essentially the same magnitude as the domestic sardines or approximately one-half of the imported volume. It is not anticipated that Gulf sardines would replace either the domestic or imported fish, but rather that the total domestic consumption would expand.

Achieving such a market share would require about 30 million pounds of canned sardines annually or about 60 million pounds on a round weight basis. In addition to the domestic market, a sizeable worldwide demand exists for sardines. Reliable estimates of worldwide supply or consumption of sardines are unavailable. However, for the purpose of this paper, it is assumed that an additional 30 million pounds of canned sardines or 60 million pounds of fish, round wieght basis, could be exported. As I pointed out in an earlier publication (2), Africa offers a potential market for U. S. fishery products. Thus, the total annual harvest of Spanish Sardines which would be required to serve both the domestic and export markets would amount to 120 million pounds or 60,000 tons. An annual harvest of that magnitude appears to be well within the estimated maximum sustainable harvest parameters.

Economic Impact of the Resource

Commercial fishermen have indicated that Spanish Sardines can be profitably produced in a large volume for a price of approximately $100 per ton. On that basis, the ex-vessel value would be $6 million annually. Based on National Marine Fisheries Service estimates (3) of Value added for all U. S. Fishery products, Table 4, that catch would yield canned sardines with a retail value of $16.6 million. Value added in the various stages of processing and marketing would amount to $10.6 million. The total impact of a $16.6 million fishery on the economy is not precisely known but should be in the area of $30-$50 million annually.

	Production Mil. lbs.	Value Mil. Dol.	Price Per lb.
1965	30	11	.37
1970	19	11	.58
1971	22	13	.60
1972	37	24	.65
1973	23	16	.70
1974	25	22	.88
1975	26	25	.96
1976	25	24	.96

TABLE 1

U. S. SARDINE PRODUCTION AND VALUE

Source: U. S. National Oceanic and Atmospheric Administration, Fishery Statistics of the United States, annual edition, 1977.

	Domestic		Imported		Total Supply	
	Mil. lb.	Pct	Mil. lb.	Pct	Mil. lb.	Pct
1965	30	40	45	60	75	100
1970	19	29	47	69	66	100
1971	22	30	50	70	72	100
1972	37	35	70	65	107	100
1973	23	25	67	75	91	100
1974	25	27	69	73	94	100
1975	26	46	31	54	57	100
1976	25					

TABLE 2

SUPPLY OF SARDINES

Source: U. S. National Oceanic and Atmospheric Administration, Fishery Statistics of the United States, 1977.

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YEAR	DOMESTIC	IMPORTED	TOTAL
---------------------- Million Dollars ----------------------			
1965	11	17	28
1970	11	27	39
1971	22	30	52
1972	24	46	70
1973	16	47	63
1974	22	61	83
1975	25	30	55
1976	24		

TABLE 3

VALUE OF SARDINES SOLD IN UNITED STATES

Source: U. S. National Oceanic and Atmospheric Administration,
Fishery Statistics of the United States, 1977.
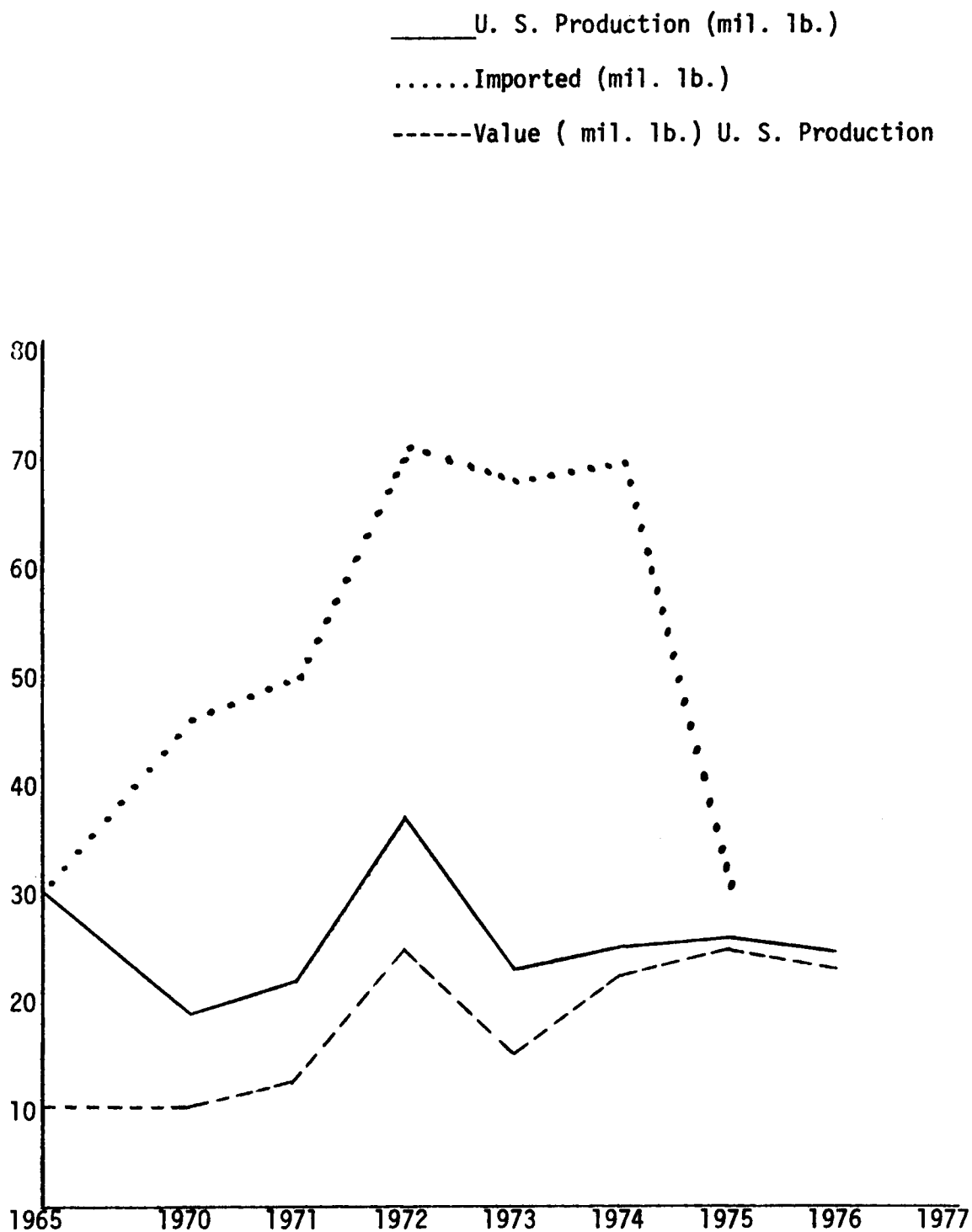
FIGURE 1

TOTAL SARDINE SUPPLY AND VALUE

Source: U. S. National Oceanic and Atmospheric Administration,
         Fishery Statistics of the United States, 1977.

	1975		1976	
	Mil. Dol.	Percent of Retail	Mil. Dol.	Percent of Retail
Domestic Landings	971	32.2	1,353	36.0
Marketing Margins Processor	861	28.6	919	24.5
Wholesale	481	16.0	602	16.0
Retail	695	23.1	880	23.4
Total Markup	2,037	67.7	2,401	64.0
Retail Value	3,008		3,754	

TABLE 4

VALUE ADDED TO SEAFOOD PRODUCTS

Source: Fisheries of the United States, 1976. Current Fisheries
Statistics 7200, April 1977.

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Sardines Processed in Mississippi

During 1977 the staff of the Food and Fiber Center of the Cooperative Extension Service of Mississippi State University processed and canned three separate lots of Sardinella anchovia. The fish varied in length from 4 to 6 inches before dressing. The fish were taken by a fisherman who produces fish commercially. All fish processed had been frozen after being caught.

An established seafood canning plant on the Mississippi Coast processed the fish. Since there are no closing machines on the Gulf Coast which are capable of closing the flat 4½ ounce sardine cans, the fish were packed in No. 303, 1 pound cans. The use of these cans required that the fish be placed in the cans in a vertical position. Even in that position the fish maintained their shape and integrity extremely well after processing, in spite of rough handling.

The fish were prepared according to well established and time-tested procedures. The fish were beheaded, eviscerated, and the tails trimmed. They were exhausted at a temperature of 220° F for ten minutes. The cans were then inverted and the liquid drained. Selected packing mediums were added to the cans which were then closed and retorted for 120 minutes at 240°F. Heat penetration data were collected to assure the proper cooking time and temperature for a safe product.

Three different packing mediums were used in processing the Gulf Sardines, soybean oil, tomato sauce, and mustard sauce. While both powdered and liquid forms of tomato and mustard were tried, the liquid form proved to be the most attractive and palatable. Results of the laboratory analysis of the sardines packed in oil is shown in Table 5.

Consumer Acceptance

A consumer taste panel was convened at Mississippi State University to evaluate the Gulf Sardines as compared to commercially available sardines. The Gulf Sardines were rated significantly higher than the commercial sardines in overall taste, texture and odor. The oil pack received the highest rating while the tomato pack was rated second.

Panelists commented favorably on the manner in which the Gulf Sardines packed in both oil and tomato sauce had a more mild odor than the commercial products packed in a similar medium.

Panelists also commented favorably on the manner in which the Gulf Sardines retained their identity and shape as individual fish.

Based on the results from the taste panel, Gulf Sardines were found to be a very desirable product with a potentially high rate of consumer acceptance.

Sardine Consumption in the United States

The per capita consumption of sardines in the U. S. is quite low, averaging only 0.4 pounds per year over the past 7 years, (Table 6). Per capita consumption declined from a high of 0.5 pounds in 1973 to a low of 0.2 pounds in 1975. The 1976 figure showed a slight increase to 0.3 pounds.

Moisture	61.0%
Protein	15.8%
Fat	21.8%
Ash	1.5%

TABLE 5

ANALYSIS OF SARDINES CANNED IN OIL

	Pounds Per Capita
1970	.4
1971	.4
1972	.4
1973	.5
1974	.4
1975	.2
1976	.3

TABLE 6

PER CAPITA CONSUMPTION OF SARDINES

Source: U. S. National Oceanic and Atmospheric Administration,
Fisheries of the United States, annual

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Demand Analysis for Sardines

Sardine consumption per capita shows a distinct inverse relationship to price, Figure 2. The estimated demand curve for sardines, Figure 3, exhibits the same general shape as a typical demand curve, sloping downward to the right. Higher prices for canned sardines during the 1970 - 1975 period have been associated with lower per capita consumption.

The demand equation for canned sardines may be written:

$$Q = a - bP$$

where:

Q = Quantity of sardines demanded on a per capita basis

a = A constant

b = Coefficient of price change

P = Price per pound

Thus, the demand equation for sardines since 1970, calculated by the least squares technique is:

$$Q = 0.67 - 0.39 \ P$$

The price elasticity of demand for sardines may be calculated as follows:

$$E = \frac{\partial Q}{P}$$

where:

E = Price elasticity

$\frac{\partial Q}{P}$ = First derivative

The price elasticity in this instance is:

$$E = \frac{\partial Q}{P} = 0.67 - 0.39 \ P$$

$$E = -0.39$$

Thus, a 0.39 percent decrease in the price of canned sardines would result in a one percent increase in the quantity demanded on a per capita basis.

Estimated Canning Costs

Since there is no current commercial processing of canned Spanish Sardines in the U. S., precise cost analysis was not possible. Estimates contained in Table 7 were based on costs associated with processing the sardines in No. 303, one pound cans based

on other types of canned fish in similar packs and using similar procedures.

To the $0.55 per can processing and transportation cost would have to be added the wholesale and retail markup to arrive at the retail selling price. Using average margins, the wholesale price would be about $0.71 and the retail price about $1.00.

Plans are currently underway to pack Spanish Sardines in a typical sardine pack.

Fish	$ 0.15
Cans	$ 0.10
Processing	$ 0.25
Transportation	$ 0.05
TOTAL per can	$ 0.55

TABLE 7

APPROXIMATE COST OF PROCESSING SARDINES
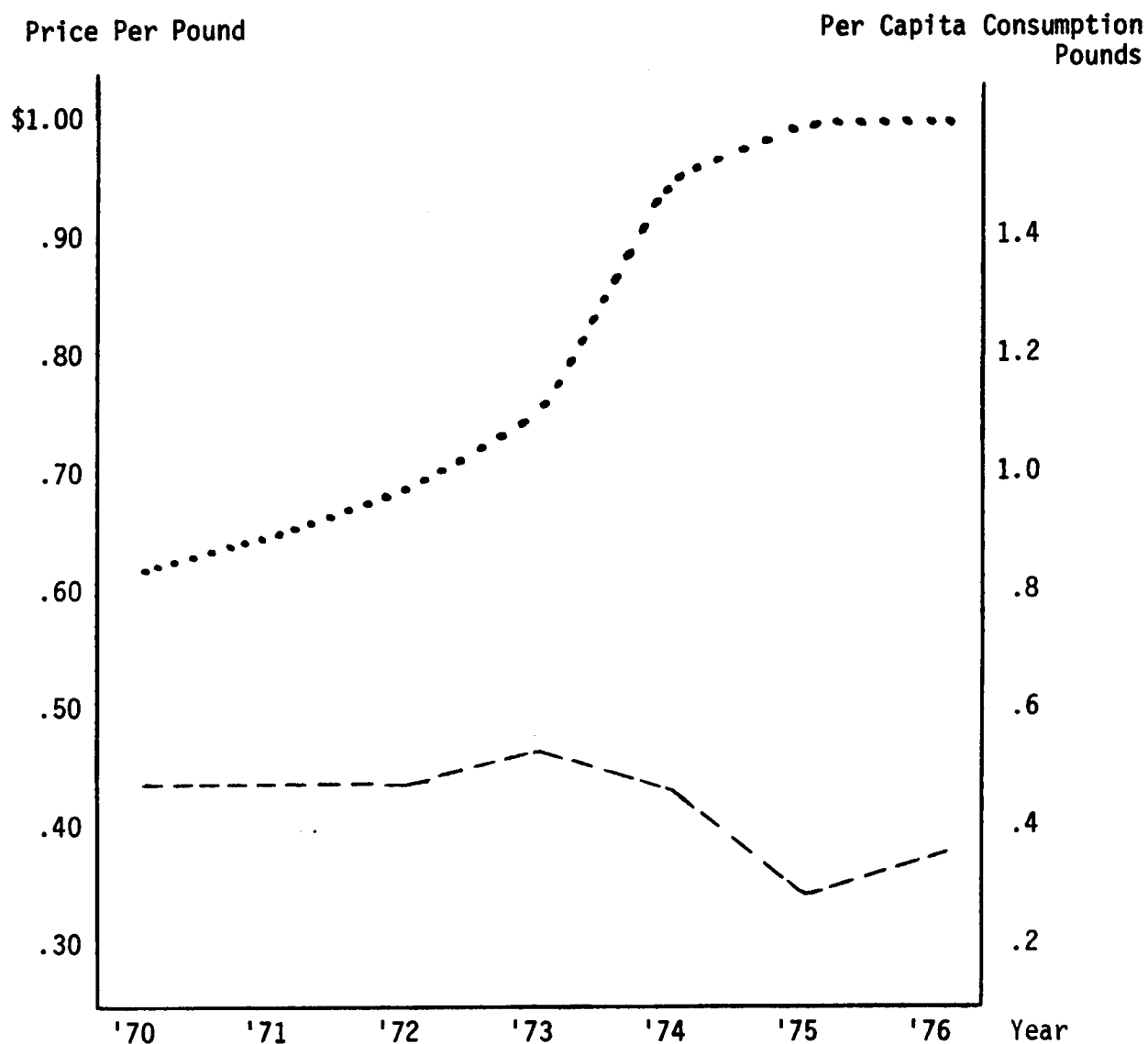
FIGURE 2

PRICE AND PER CAPITA CONSUMPTION
OF SARDINES, 1970 - 1976
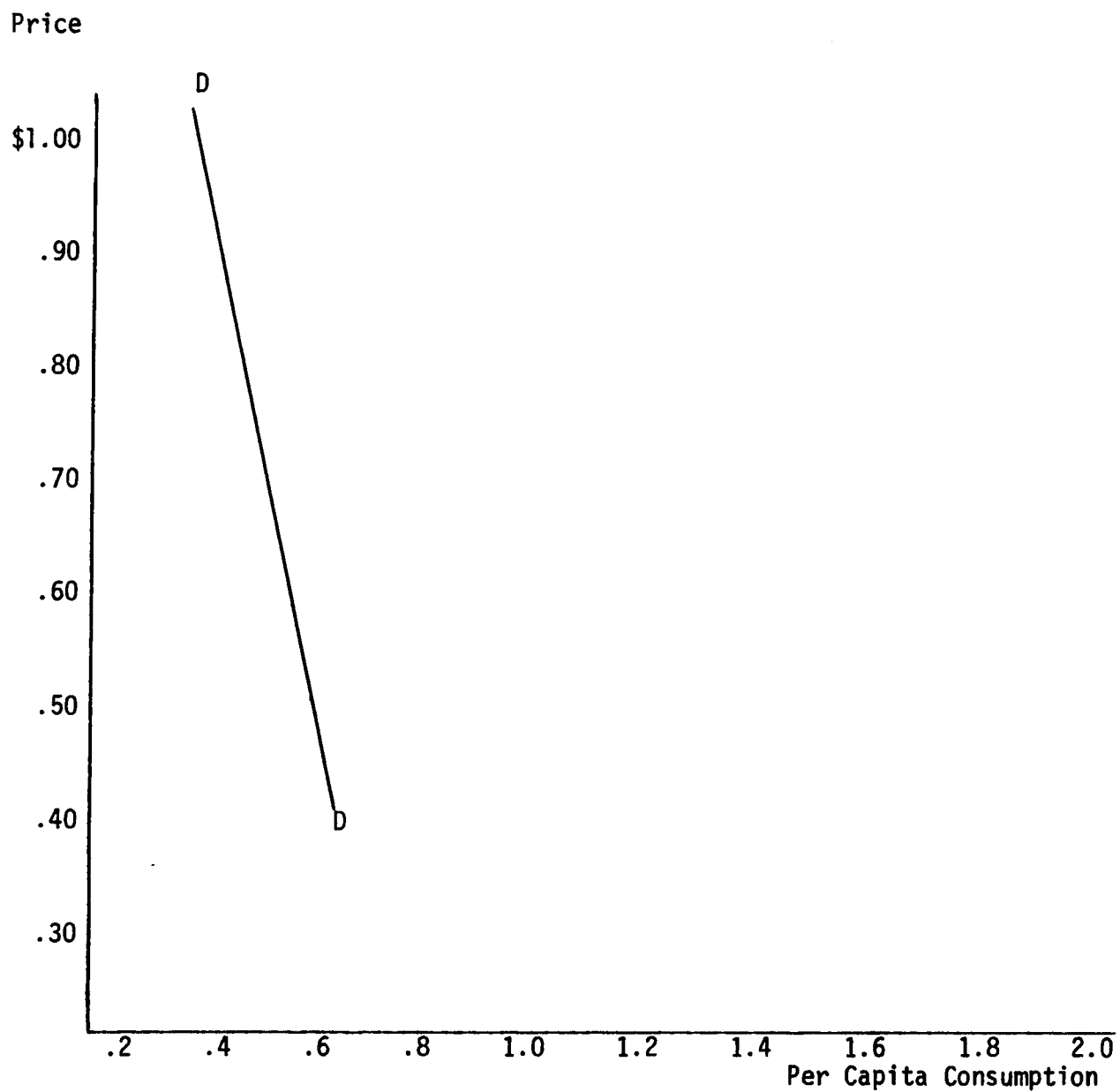
FIGURE 3

DEMAND CURVE FOR SARDINES

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ECONOMIC PROGRAMS TO STIMULATE
UTILIZATION OF GULF OF MEXICO GROUNDFISH

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Numerous species of groundfish and pelagic fishes in the Gulf of Mexico have gone unutilized despite their abundance and value as a source of additional protein. Several studies have documented the abundance of these species (4, 8, 11) and their specific composition (14). The marketing aspects in the utilization of these fishes have also been investigated (1, 10, 2) as well as the economics of harvesting such fishes (12). Despite this past research, utilization is still nonexistent except in isolated cases, e.g., use in pet food.

Questions must be raised as to whether this research has adequately addressed the question of why has not utilization taken place at greater levels. The purpose of this paper is to analyze the question of nonutilization of Gulf of Mexico groundfish. It is precisely this question which is the key element and final link with previous research. The paper also proposes steps to remedy this situation in terms of both the public and private sectors. The remainder of the paper is divided into three sections: a methodological framework to explain the low level of groundfish utilization, a discussion on programs to stimulate greater exploitation of groundfish, and a summary and conclusion.

METHODOLOGICAL FRAMEWORK

While there is general agreement on the substitutability of sciaenid and other species for fish products from cold-water species (13, 9), we find greater pressures on preferred cold-water species, resulting in increasing prices, and over-capitalization in these fisheries. On the other hand, the harvesting sector cites inadequate demand, and, primarily, lower potential profits from warm-water species. Despite the benefits to society from the availability of lower-cost protein substitutes and reallocating inefficient resources to the Gulf, these developments will not occur when decisions are based solely on private (industry's) benefits and costs.

The distinction between private and public benefits and costs enables us to analyze the problem of not fully utilizing Gulf of Mexico fishery resources. In the framework of externalities created in the consumption and production of commodities, it becomes apparent

why private industry at times does not supply the socially optimal amount of the commodity desired.

Externalities can be defined as unintended side effects, beneficial or harmful, from the act of producing or consuming a good. Externalities are felt between and among producers and consumers. A good example is the particulate matter emitted by heavy industry; if the pollution is allowed to continue unabated, then society at large bears the cost. Thus, this externality, if internalized in the firm, would result in less produced when both private and social costs are aggregated.

An illustration of this process can be examined through Figure 1. Here we have hypothetical demand and supply curves for sciaenids and other groundfish in the Gulf of Mexico. The intersection of demand and private marginal costs (supply) determine an output level of Q_c. However, if we assume that a positive externality to society is created from supplying groundfish -- through reduced seasonal unemployment, less pressure on other fisheries -- then there is also an externality curve 'E'. Society's "true" supply curve is the subtraction of E from private marginal costs, or curve SC. Now, the intersection of SC and demand yields the optimal amount desired, Q_s.

In a similar vein, there may be negative externalities created from supplying certain fish products, especially those species with heaving fishing pressures -- redfish in particular (17). Here, the externality curve in Figure 2 must be added to private cost. This aggregation of externality costs and private costs, curve SC, now results in an optimal quantity on the market (Q_s) less than the quantity Q_c when only private costs were considered. In both cases, above the positive externalities, and the negative externalities at hand, the proper quantities of both commodities are consumed and produced.

For externalities associated with consumption, rather demand, positive externalities are added onto the demand curve and negative externalities are subtracted from it -- just the opposite from above. There may be goods with externalities associated with both production and consumption. Gulf of Mexico groundfish and pelagic species may be one such good, where a lower cost protein substitute is available for consumption and production may relieve over-capacity in other Gulf fisheries. The problem in attaining the socially optimal quantity is recognition of externalities, and once recognized, proper steps by both the public and private sectors to achieve that quantity.

DISCUSSION

In this section we discuss programs to insure that the optimal quantity of groundfish and pelagic species from the Gulf of Mexico is produced and consumed. It is our contention that positive externalities are associated with the production and consumption of these fishes, a condition which justifies possible intervention by the public sector in the market place. Here, programs are classfied into two areas: programs stimulating the demand for these fishes, and

programs designed to increase the supply of these fishes and resulting products. The list of programs is by no means exhaustive but rather serves as a starting point for discussion.

Demand Side

The problem of stimulating demand could be addressed in both the domestic and foreign markets. In the domestic market, a program of government (federal and/or state) contracts to purchase fish products could be established. The fish products could be served in mass feeding situations, i.e., primary and secondary schools, colleges, hospitals, the military, and other government institutions which prepare meals for employees or a clientele. Specifications should be drawn up which insure proper quality and characteristics of the product. Rough guidelines on prices for these products at various marketing levels have been established in the aforementioned research (1, 10, 2, 7). We envision that initially contracts be from institutions in proximity to the Gulf, yet at quantities which would spur fishing and processing activity.

This program of government contracts to purchase fish products can have multiple effects. First, overall demand for these species is increased through the contracts themselves. Secondly, through introduction and awareness of new fish products the increase in demand may experience "multiplier effects", i.e., growing consumer demand may encourage purchases by the private sector. Both developments, public and private buying, would have the reinforcing effect on the industry to supply more of these species.

In relation to foreign markets, the public and private sectors could cooperate on joint research to fathom consumer demand abroad. Foreign, as well as domestic, markets for Gulf of Mexico groundfish and pelagic species should be considered. The potential amount of products that could be processed from Gulf of Mexico sciaenids and other species is so large that allocation to the domestic market alone would significantly increase fish consumption and the composition of fish demand. Therefore, foreign markets should receive serious consideration.

Consumer demand for finfish in the United States is characterized by the preference for species with white meat which flakes easily. This preference is so pronounced, in fact, that the U.S. imports at least double its domestic catch of cod, haddock, flounder, and pollock. Unfortunately, these characteristics are common to only a very few of the species under question. However, the demand for warm-water species is often overlooked in other countries. This is all the more an oversight because of the burgeoning populations and expanding economics in the nations of Asia and Africa.

Two factors in particular point to the potentiality of foreign markets. First, experience with most agricultural commodities indicate proportionately greater export sales can occur when price is lowered. Known as price discrimination (3), the fishing industry can optimally allocate quantities of fish products into both domestic and foreign markets depending on the nature (elasticity, c.f.6) of their respective

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demands. Second, passage of the Fisheries Management and Conservation Act (P.L. 94-265) protects Gulf fishery resources for the American fishing industry. This resource will prove to be of increasing value over time as domestic and foreign demand for high-protein foods increases.

Supply Side

On the supply side, programs to help lower the private marginal costs of supplying fish products would increase fishing activity towards the species under question. The programs we envision are primarily aimed at injecting more capital into the industry and lowering the fixed costs of vessel ownership. Thus, these programs only affect the industry indirectly, much unlike other government programs which give subsidies to producers, sets transportation rates, and sets quotas on imports.

With respect to injecting more capital into the industry, many firms could take advantage of either a limited partnership or a joint ventures type of organization. Both offer unique advantages to firms and investors. In this regard, the government's role could be one of facilitating the perception of firms and investors of each other and the benefits one can do the other. Investors can be made aware of opportunities with a growing industry and conversely fishing firms can seek out the appropriate segments of the economy for captial with the proper training.

By definition, limited partnerships are composed of one or more general partners and one or more limited partners, with the assets of the general partner liable for the debts of the limited partnership. Each limited partner's liability is limited to his investment, so that his personal assets cannot be reached to satisfy the financial obligations of the limited partnership.

From the general partner's prospective, a major purpose of the limited partnership is to raise equity capital. A good case in point has been the cattle feedlot limited partnerships which grew with the demand in fed beef (5). The general partner, however, should be aware of state and federal registration requirements, brokerage commissions, and all offering expenses. From the limited partner's perspective, investors are usually interested in (1) limited liability, (2) some assurance that the investment is readily marketable at all times, (3) income tax minimization and/or management, as well as (4) rate of return on investment.

While regulations vary somewhat from state to state, generally limited partners cannot participate in management decisions nor can their names be used as a part of the limited partnership name. Observance of these formalities will guarantee that the limited partners receive limited liability. In addition, if the general partner is incorporated, then only corporate assets may be used to satisfy partnership debts and obligations. Thus, a limited partnership may organize so that limited liability is provided for all investors.

The second program to consider is joint ventures. Joint ventures

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provide a means whereby two or more business organizations (partnerships, corporations or cooperatives) can form an association to carry on a specific business activity and still retain their own identity. Individual participants share, on an agreed basis, the expenses, profits, losses and some measure of management control over the conduct of the ventures operation. For income tax purposes, joint ventures are treated as partnerships. The motivating force behind the creation of joint ventures can be quite varied depending upon the needs of the participants. The more common ones being: gaining access to the consumer markets, securing potential price premiums, pooling of financial resources, and realizing economies of scale. Joint ventures are particularly attractive in development of new products where the fishermen and processors are unable to individually develop the market.

The advantages of joint venture operations follow closely those of the common law partnership, and also incur many of the same disadvantages. The key to any joint venture is an equitable agreement between the parties outlining their duties and obligations. The terms ofownership should clearly specify each parties' claim to the venture's equity and capital assets. To prevent domination by one party and conflicts due to differing goals, the voting rights and arbitration procedures in making joint decisions should be specified. The more equal the participants, the better the chances for success.

Arrangements for credit procurement and exploitation of each other's financial and management resources must be made. Cash flow problems created by the transfer of intermediate products can be handled with formula pricing.

With joint ventures generally being created to engage in a new activity there will be some changes in the risks that each participant faced. The increased risks or the change in the composition of risk can often be offset by cost savings, insured supplies or markets, quantity control, etc.

Joint ventures formed with foreign corporations to market U.S. catches should explore all the areas of possible conflict between the firms before joining together. Advice from the International Section of some of the larger commercial banks should be particularly helpful. One should not overlook contract agreements as an alternative marketing arrangement to joint ventures.

The last program on the supply side is a proposal for modifications in the Federal government's Fishing Vessel Capital Construction Fund and Obligation Guarantee Programs. While there is ample description of these programs elsewhere (15, 16), we recommend modifications in these programs so that firms and fishermen planning to exploit underutilized species find the programs more accessible.

At present, the Capital Construction Fund and Obligation Guarantee are available to fishermen and firms in all fisheries. However, there is a provision whereby these programs may not be available to "conditional" fisheries, i.e., those fisheries determined to have excess vessel capacity in terms of the available resources. Because of changing

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conditions in many fisheries, and political pressures, no fishery is classified as such. Due to this problem, we recommend an alternative approach-classification of under-utilized species as having priority for these programs. In this way, government efforts, which should reflect society's desires, are directed towards fisheries most in need.

SUMMARY AND CONCLUSIONS

In this paper we have attempted to explain the low-level of utilization of Gulf of Mexico groundfish and pelagic species. The theoretical framework for the analysis was in terms of private and public benefits and costs which involve externalities. Viewed in this perspective, it becomes apparent why private industry sometimes fails to provide the optimal amount of a commodity. In our analysis, we identified Gulf of Mexico groundfish and pelagic species as those goods which have positive externalities associated with their consumption and production.

The second half of the paper discusses measures with which the optimal amount of the desired species can be demanded and supplied. Thus, programs designed to work on the demand and supply side of the market were suggested. The programs are actions to be taken by the public sector (government) which may also have secondary, and positive, effects on the private sector. In this way both the public and private sectors can work hand in hand at a balanced use of our fishery resources.
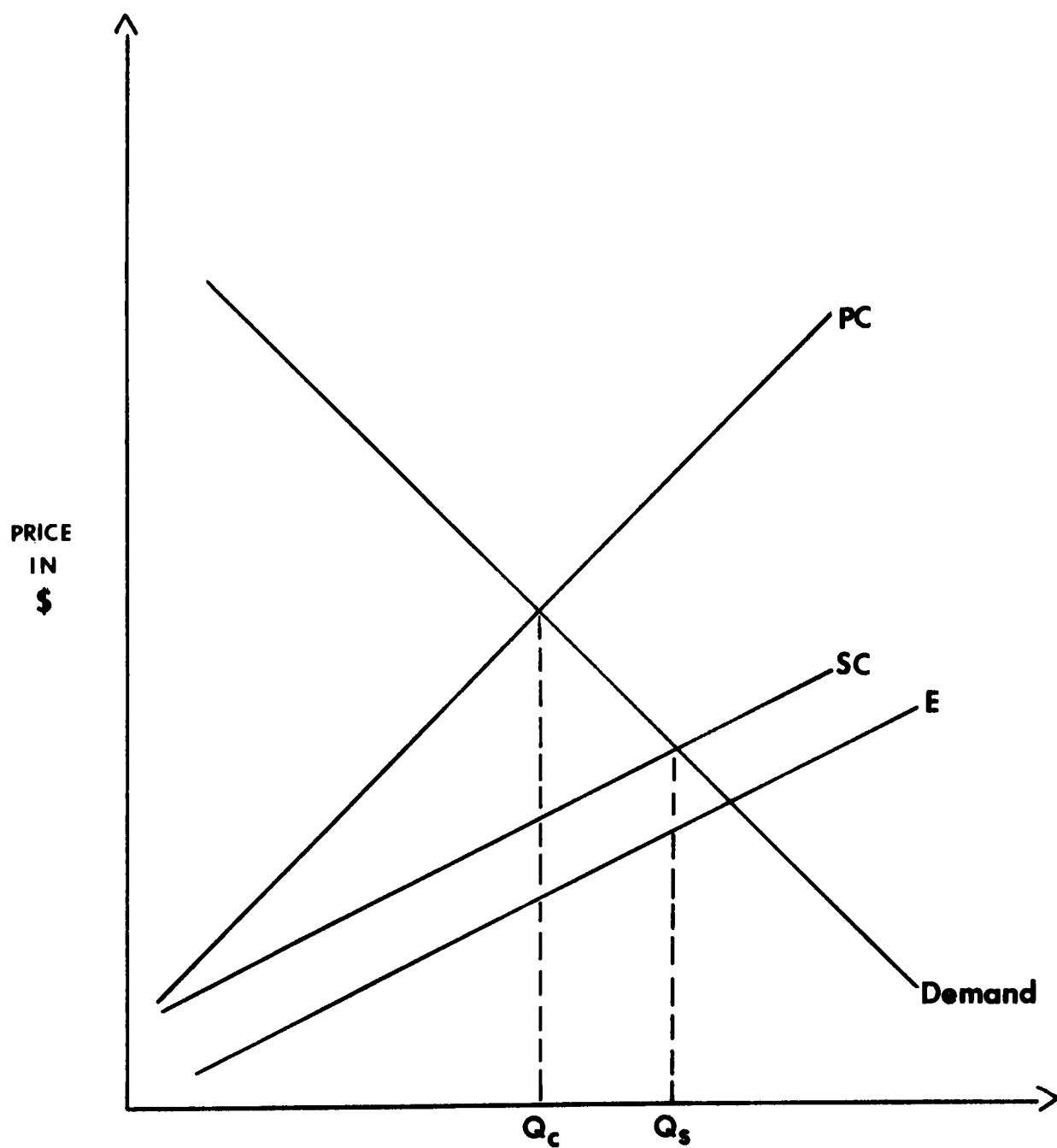
FIGURE 1. EXAMPLE OF POSITIVE EXTERNALITY
ASSOCIATED WITH PRODUCTION OF
A COMMODITY
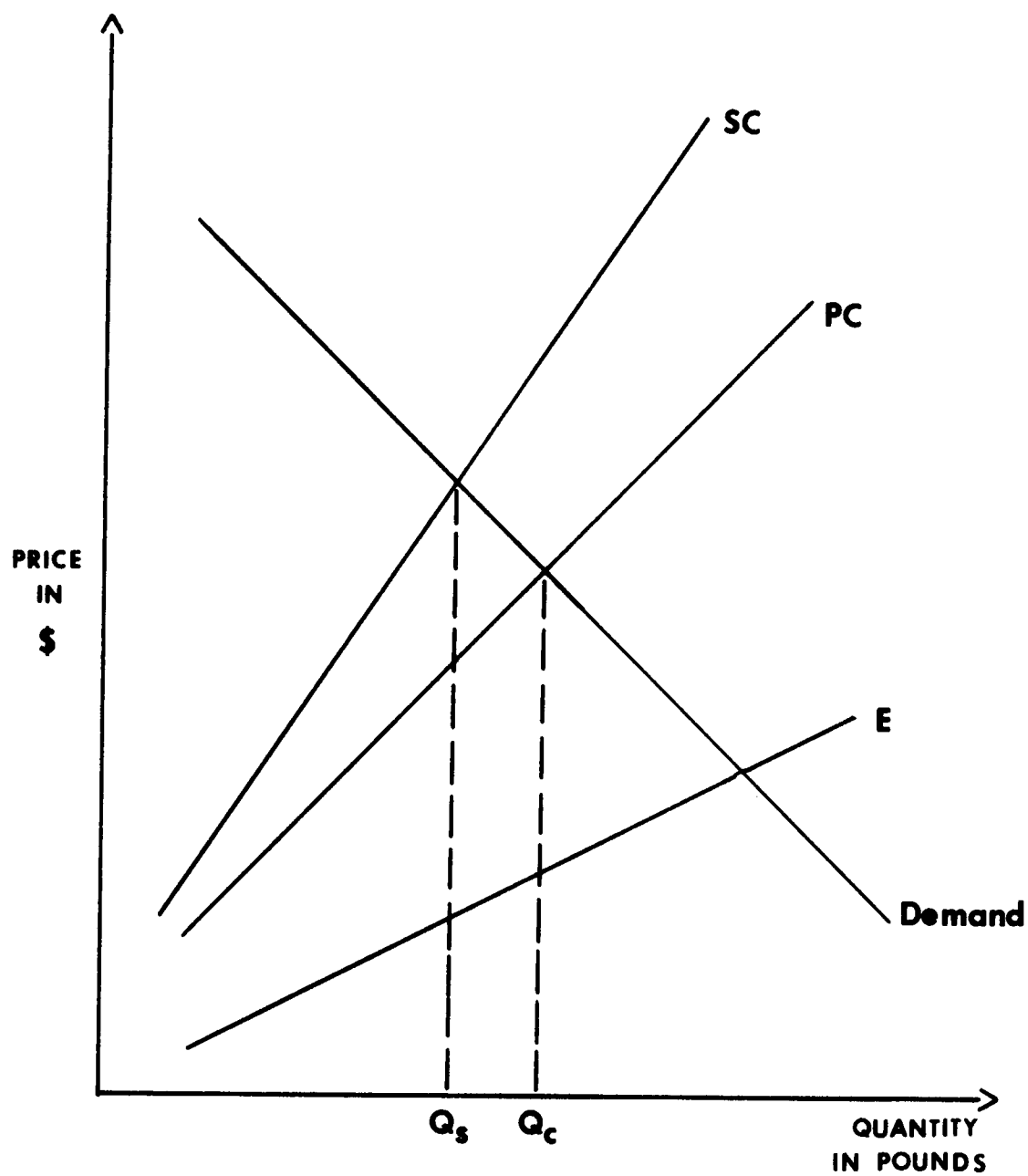
FIGURE 2. EXAMPLE OF NEGATIVE EXTERNALITY
ASSOCIATED WITH PRODUCTION OF
A COMMODITY

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EFFECTS OF DIFFERENT SPECIES OF CRUSTACEANS AND SPOILAGE CONDITIONS ON THE CHEMICAL AND MOLECULAR WEIGHT CHARACTERISTICS OF CHITIN/CHITOSAN PRODUCTS

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While many potential useful applications are being explored for chitin and chitosan, the control of the product quality becomes important for producing the right product for the right application. Varieties of chitosan products can be produced by varying the manufacturing conditions, such as means of deproteination, time, temperature, atmosphere, and the concentration of the alkaline solution used in the deacetylation step etc. (1,2). Another variable investigated in this study was the effects of using different raw materials upon the quality and characteristics of chitosan products.

Exoskeletons of different crustaceans species contain 20 to 70% chitin and various amounts of minerals, and protein (6). Ash contents varying from 25% for shrimp to 55% for crab have been observed in this laboratory. Yet, questions remain as to whether chitin and chitosan products from different species are different from each other in terms of degree of deacetylation and polymer molecular weight. Findings in this area concerning whether skeletons of mixed species can be used for producing chitin and chitosan products will facilitate the supply of raw material for chitin or chitosan. Other factors, such as the freshness of the hulls and chemical preservatives were also speculated to affect the quality of the product. Although chitin had been recognized as a very stable polymer, hydrolysis or deamination might occur due to the effects of microbial spoilage on the shells (7).

Thus, this study was designed to investigate the possible product variations caused by the choice of different species and by the spoilage of hulls at ambient temperatures.

MATERIALS AND METHODS

Raw materials

Shrimp shells from three different species (white, pink, and brown) were obtained from the King Shrimp Co., Inc. (Brunswick, Georgia). Samples collected for species consisted of three different lots collected on different days, and frozen immediately after collection. Three batches each of fresh and dried hulls from the blue crab were obtained by the Lewis Crab Factory, Inc. (Brunswick, Georgia). The dried samples were obtained from a commercial renderer using a flame dryer. The crayfish were collected by one of us (JRS) from Ohio streams, dried as below, and shipped to us in Georgia. The Alaskan Seabob shrimp and Tanner crab hulls were supplied by Bio-Dry, Inc. (Kodiak, Alaska) from their commercial rendering process. Two batches of brown shrimp, obtained locally were stored at ambient temperature for 16 days and served as the spoiled samples to be compared with two batches of control hulls freshly frozen.

Manufacturing of chitin and chitosan

Wet hulls were first dried by a convection oven at 103 C for 24 hours. They were then ground by a Wiley mill to pass through the screen with 1 mm openings. Each sample was then subjected to the same procedure of demineralization, deproteination, and deacetylation as described in the previous study (2). The brief summary of the procedure was as follows: (i) demineralization at room temperature for 30 minutes with a 10% excess of HCl in 0.5N concentration over the stoichiometric amount of the ash content in dry hulls; (ii) deproteinated with ten volumes of 1% (w/w) NaOH, in relation to dry weight of hulls, for one hour at 65 C, washed and dried at 85 C (chitin was thus obtained); (iii) deacetylated with 50% (w/w) NaOH at 100 C for 2 or 5 hours in a 4-liter reaction kettle purged with nitrogen gas. The chitosan product thus obtained was washed and dried overnight for 18 to 20 hours at 70 C.

Viscosity measurement

The preparation of the chitosan solution on which viscosity was measured involved wetting with distilled water followed by adding anhydrous acetic acid to make a 2% solution. The chitosan solution was prepared in 10% (w/w) net concentration on a dry and ash free basis. Final volume of the preparation was 500 ml. The temperature was brought to 20 C before viscosity was measured with a Brookfield model RVI spindle type viscometer.

Moisture and ash

They were determined according to A.O.A.C. methods (4). These data were needed for adjusting the amounts of reagents required in the manufacturing process, the preparation for viscocity measurement, and the calculation of acetyl contents.

Acetyl group determination

The amine free titration method was used for sol-

uble chitosan samples based on the method of Broussignac
(3). The method of Lemieux and Purves (5) was used for
analysis of acetyl contents in the chitin samples. The
data were expressed as percentages and then converted to
percent deacetylation values based on the number of de-
acetylated glucosamine units out of the total number of
monomner units (2).

Molecular weight distribution (MWD)

High pressure liquid chromatography (HPLC) with
Glycophase-CPG columns (Water Associate, Mass.) was used
for determining the MWD. Details of the method were
described by Wu et al. (8) and Bough et al. (1). The
sample was prepared by dissolving a 5% (w/v) quantity
of chitosan sample in 0.33 M acetic acid which was fol-
lowed by adding sodium acetate to make 0.2 M concentra-
tion.

Statistical analyses

Differences among samples in terms of viscosity,
acetyl content, and MWD were analyzed by the analysis
of variance and the Duncan's multiple range test. Only
samples from white, pink, and brown shrimp, and blue
crab were tested statistically. Alaskan Seabob shrimp
and Tanner crab, and crayfish had only one batch per
species, and thus, they were not analyzed statistically.
Data of these samples were reported based on the ave-
rage of duplicate determinations. Comparison of fresh
vs. spoiled shrimp hulls was based on duplicate deter-
mintaions on duplicate samples from one lot of the
brown shrimp sample.

RESULTS AND DISCUSSION

Chitin product

The acetyl contents of different chitin products
ranged from 17 to 19% acetyl or 12 to 22% degree of de-
acetylation (%DA) as shown in Table 1. The sample from
white shrimp had 19.04% acetyl or 12.45% DA (percent of
monomer units being deacetylated), which was significantly
(P<0.01) less deacetylated than the rest of the samples
tested. Whereas, the sample from commercially dried
blue crabs gave 17.73% acetyl or 19.54% DA, which was
significantly (P<0.01) more deacetylated than the other
samples from pink shrimp, brown shrimp, and fresh blue
crab. Their acetyl contents were approximately 18% and
similar values were observed for percent deacetylation.
The acetyl contents for crayfish, Alaskan Seabob shrimp,
and Tanner crab were 17.72, 17.19, and 17.89% respectively,
which were equivalent to 19.81, 22.69, and 18.86% degree
of deacetylation, respectively. These results indicated
that although shrimp and crab shells consist of different
amounts of protein, mineral and chitin, their purified
chitin products are similar in terms of degree of de-
acetylation. The differences among lots within a species
were as great as the differences among different species.

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Other differences, such as chitin molecular size of mac-
cromolecular structure, were not investigated in this
study.

Chitosan products

In a previous study on chitosan manufacturing var-
iables, it was found for the conditions tested (50% NaOH
at 100 C) that the exponential phase of deacetylation
which was completed after two hours, and longer deacety-
lation times, only led to a slight increase in the degree
of deacetylation (2). Thus, comparison of the chitosan
products was carried out based on the products deacety-
lated for two-hours, which had been exposed only to the
optimum amount of treatment, as well as on the products
deacetylated for five-hours which had been exposed to
the more extensive deacetylation treatment. Products
were compared based on their degree of deacetylation,
viscosity, and molecular weight distribution. The degrees
of deacetylation of different products as shown in Table
2 ranged from 75.5 to 78.9% for two-hour samples as ex-
pected (2). There were no statistical differences (P<
0.01) among the chitosan products prepared from the five
species of shrimp and crab where the products were de-
acetylated for the same length of time. The slight dif-
ference in the degree of deacetylation detected in the
chitin products of white shrimp and commercially dried
blue crab vs. chitin from the rest of the species could
no longer be detected by the free amine analysis after
chitin was converted to chitosan through the relatively
harsh treatment of the deacetylation step.

Viscosity data of chitosan solutions as shown in
Table 3, from 179 to 405 cps for two-hour samples and
107 to 169 cps for five-hour samples. Viscosity as well
as the molecular weight values decreased as expected
when the deacetylation time was extended (2). The dif-
ferences in viscosity among different chitosan samples
were not statistically significant (P<0.05), partially
because complex factors were involved in the viscosity
measurement (1) and the variation among different lots
of samples in a species was great.

As shown in Table 3, the molecular weight data of
samples deacetylated for two-hours from 715,000 to
1,118,000 for $\bar{M}_w$, 196,000 to 311,000 for $\bar{M}_w$, and 3.6 to
7.5 for dispersity. Corresponding values for five-hour
samples were 445,000 to 784,000 in $\bar{M}_w$, 87,000 to 214,000
in $\bar{M}_n$, and 3.5 to 5.1 in dispersity. The result of
Duncan's multiple range test at 1% probability is shown
in Table 3 for samples from five species. In general,
products from white shrimp, pink shrimp, and fresh crab
showed no significant differences in polymer molecular
weight values, whereas the product from brown shrimp
had a significantly higher molecular weight and com-
mercially dried blue crab led to a product with lower
molecular weight (P<0.01). Whether the low molecular

weight and high degree of deacetylation observed on
the sample from the commercially dried blue crab is due
to the drying process employed is undetermined. There
was no significant difference (P<0.01) in the dispensity
values for all samples. Samples from crayfish and Alas-
kan Tanner crab, has surprisingly high molecular weight
values as shown in Table 3, as compared with other sam-
ples.

Fresh hulls vs. spoiled hulls

After 16 days storage at ambient temperature, the
hulls from brown shrimp showed intensive putrefaction.
Although the hull was still intact, no other obvious signs
of decomposition on the shells were observed. Two batches
each of the freshly frozen and spoiled hulls were used
to produce chitin and chitosan products using the same
manufacturing procedure. The qualities of the products
were compared based on the degree of deacetylation (Table
4), viscosity, and molecular weight data (Table 5).
Statistical analyses showed that there was no significant
difference (P<0.05) in degree of deacetylation between
chitin samples produced from fresh and spoiled hulls.
No other characteristics were examined for these chitin
products in this study. Differences between the degree
of deacetylation, viscosity, or molecular weight data of
the two-hour chitosan products from the fresh and spoiled
hulls were also not significant (P<0.05). The variance
among the two-hour chitosan products and results of det-
ermination techniques may simply overshadow the insign-
ificant difference caused by the spoilage treatment.
For five-hour chitosan products, there were still no
significant differences (P<0.05) in degree of deacetyl-
ation or viscosity data between products from the fresh
and spoiled samples. Yet, the poylmer molecular weight
values of the product, 717,000 for $\overline{M}_w$, and 177,000 for
$\overline{M}_n$ were significantly higher (P<0.05) than those of the
products from the spoiled hulls, 592,000 $\overline{M}_w$ and 134,000
in $\overline{M}_n$. Therefore, we failed to prove that there is a
difference in degrees of deacetylation between the spoiled
and unspoiled samples in this study, while the polymer
molecular weight of the spoiled sample was found decreased.
Whether this is due to the hydrolysis of the polymer during
the spoilage or due to the change of physical structure
of the hulls, thus affecting the penetration rate of
alkali solution used in the deacetylation step is un-
determined.

CONCLUSION

In general, this study suggests that shells from
different species of shrimp can be converted into chitin
chitosan products with little difference in degrees
of deacetylation, viscosities, and molecular weight
values. The product from the fresh crab shells is com-
parable with that produced from shrimp hulls. Products

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differences brought about by species differences seems
to be insignificant as compared with the difference
created by the different processes employed, such as
varying the deacetylation time as in this study, or
other manufacturing conditions as in previous studies
(1,2).

Spoilage for two weeks does not seem to create any
significant difference in the acetyl contents of its
chitin or chitosan products, but it causes the decrease
in the molecular weight of the chitosan product deacet-
ylated for five-hours by more than 15%, although the
effect is not apparent for the product deacetylated for
a less extent. It is suggested that the spoilage of
hulls should be kept to a minumum, not only for sani-
tation purposes, but also for maintaining the quality
of the product.

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TABLE 1

CONTENTS OF ACETYL GROUP IN CHITIN SAMPLES PRODUCED FROM HULLS OF DIFFERENT CRUSTACEAN SPECIES

Samples	% Acetyl[1]	Ranks[2]	%DA[3]	Ranks[2]
White Shrimp	19.04 ± 0.42	a	12.45 ± 2.35	d
Pink Shrimp	18.07 ± 0.52	bc	17.85 ± 2.90	ab
Brown Shrimp	17.76 ± 0.83	bc	19.54 ± 4.53	abc
Blue Crab	18.38 ± 0.51	b	16.14 ± 2.85	abcd
Blue Crab (commercially dried)	17.73 ± 0.39	c	19.73 ± 2.11	a
Seabob Shrimp	17.19 ± 0.42	---	22.69 ± 2.26	---
Tanner Crab	17.89 ± 0.07	---	18.86 ± 0.39	---
Crayfish	17.72 ± 0.24	---	19.81 ± 1.31	---

[1] Percent acetyl represent the fraction of MW that is contributed by acetyl groups in a chitin polymer.

[2] Samples designated by same letters have no significant difference from each other at 99% confidence level by Duncan's Multiple Range Test. Last three samples did not have enough batch replications to be tested.

[3] Percent deacetylation (%DA) represents the ratio of the number of deacetylated monomers in total number of monomer units in a chitin polymer.

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TABLE 2

DEGREES OF DEACETYLATION AND CONTENTS OF FREE AMINE GROUPS IN CHITOSAN
SAMPLES PRODUCED FROM HULLS OF DIFFERENT CRUSTACEAN
SPECIES BY DEACETYLATING FOR TWO & FIVE HOURS

Samples	Two Hours		Five Hours	
	% FA[1]	% DA[2]	% FA[1]	% DA[2]
White Shrimp	7.45 ± 0.51	78.94 ± 4.51	7.78 ± 0.35	81.90 ± 3.04
Pink Shrimp	7.27 ± 0.18	77.4 ± 1.57	7.68 ± 0.19	81.01 ± 1.67
Brown Shrimp	7.43 ± 0.12	78.83 ± 1.06	8.05 ± 0.39	83.30 ± 3.91
Blue Crab	7.42 ± 0.15	78.77 ± 1.38	7.93 ± 0.19	83.18 ± 1.87
Blue Crab (commercially dried)	7.35 ± 0.27	78.16 ± 2.42	7.53 ± 0.37	79.39 ± 3.56
Seabob Shrimp	7.05 ± 0.08	75.47 ± 0.76	7.45 ± 0.35	79.03 ± 3.19
Tanner Crab	7.08 ± 0.22	75.66 ± 1.97	7.88 ± 0.11	82.81 ± 1.01
Crayfish	7.08 ± 0.13	75.74 ± 1.19	7.72 ± 0.25	81.43 ± 2.22

[1]Percent free amine (%FA) represents the fraction of MW that is contributed by deacetylated amine groups in a chitosan polymer.

[2]Percent deacetylation represents the ratio of the number of deacetylated monomers in total number of monomer units in a chitosan polymer.

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TABLE 3

VISCOSITY AND MOLECULAR WEIGHT DISTRIBUTION DATA OF CHITOSAN SAMPLES PRODUCED FROM HULLS OF DIFFERENT CRUSTACEAN SPECIES BY DEACETYLATING FOR TWO AND FIVE HOURS, RESPECTIVELY. $\bar{M}_w$ AND $\bar{M}_n$ ARE WEIGHT AND NUMBER AVERAGE MOLECULAR WEIGHT, RESPECTIVELY

	Viscosity[‡] (cps)	$\bar{M}_w$ (10^3)	$\bar{M}_n$ (10^3)	D[**]
1. Two-Hour Samples[*] :				
White Shrimp	287^a	855^{abc}	155^b	5.5^a
Pink Shrimp	300^a	1030^a	138^{bc}	7.5^a
Brown Shrimp	221^a	1003^{ab}	226^a	4.4^a
Blue Crab	405^a	888^{abc}	131^{bc}	7.0^a
Blue Crab - CD	230^a	715^d	121^c	5.9^a
Seabob Shrimp	179^-	943	213	4.4^-
Tanner Crab	378^-	1108	311	3.6^-
Crayfish	318^-	1085	196	5.6^-
2. Five-Hour Samples[*] :				
White Shrimp	169^a	638^{ab}	127^b	5.1^a
Pink Shrimp	158^a	573^a	111^a	5.1^a
Brown Shrimp	129^a	760^{bc}	195^a	3.9^a
Blue Crab	140^a	500^{bc}	103^{bc}	4.8^a
Blue Crab - CD	129^a	445^c	87^c	5.1^a
Seabob Shrimp	107^-	706^-	168^-	4.2^-
Tanner Crab	126^-	756^-	214^-	3.5^-
Crayfish	162^-	784^-	163	4.8^-

[‡]Measured with Spindle #1 at 20 RPM   [§]Commercially dried

[*]Refering to the time of deacetylation   [**]Dispersity = $\bar{M}_w/\bar{M}_n$

[#]Samples designated by same letters have no significant difference ($P < 0.01$) in the column. Data superscribed by "_" are not tested statistically.

TABLE 4

DEGREE OF DEACETYLATION OF CHITIN AND CHITOSAN SAMPLES
PRODUCED FROM FRESH AND SPOILED HULLS OF BROWN SHRIMP

	Deacetylation Time(hrs)	Fresh	Spoiled
Chitin	0	16.54 ± 1.82	13.88 ± 2.06 (ns)*
Chitosan	2	75.56 ± 1.68	75.35 ± 1.84 (ns)*
Chitosan	5	81.18 ± 1.20	78.56 ± 3.94 (ns)*

*ns = no significant difference ($P < 0.05$)

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TABLE 5

VISCOSITY, WEIGHT AVERAGE MOLECULAR WEIGHT ($\overline{M}_w$), NUMBER AVERAGE MOLECULAR WEIGHT ($\overline{M}_n$), AND DISPERSITY (D), OF CHITOSAN SAMPLES PRODUCED FROM FRESH AND SPOILED HULLS OF BROWN SHRIMP

	Two-Hour[1]		Five-Hour[1]	
	Fresh	Spoiled	Fresh	Spoiled
Viscosity[2] (CPS)	222 ± 113	201 ± 45 (ns)[3]	159 ± 43	154 ± 17 (ns)
$\overline{M}_w$ (10³)	875 ± 157	885 ± 58 (ns)	717 ± 71	592 ± 31 (*)
$\overline{M}_n$ (10³)	193 ± 43	148 ± 3 (ns)	177 ± 28	134 ± 6 (*)
D	4.59 ± 0.2	6.0 ± 0.3 (ns)	4.09 ± 0.3	4.42 ± 0.04 (ns)

[1]
Time of deacetylation

[2]
Measured with Spindle #2 at 100 RPM under the conditions described in Methods section.

[3]
In parenthesis, ns = no significant difference, * = significant difference (P < 0.05)

FLUSHING OF SHRIMP HEADS FROM COASTAL WATERS

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During a meeting with Mr. J. Leonard Ledbetter (Director of the Georgia Department of Natural Resources, Environmental Protection Division) on November 17, 1977, concerning the results of a shrimp packing house screening study performed by the Marine Extension Service, the ensuing discussion revealed the necessity for another, related project. Specifically, it was decided that an assessment of tidal flushing of shrimp heads (discharged by packing houses) from the East River (Oglethorpe Bay) would be performed during Georgia's spring shrimp season.

However, during the middle two weeks of December, 1977, a brief period of unseasonably cold temperatures transpired which drove the shrimp out of the estuaries and into deeper, warmer waters. These conditions made shrimping possible during a period in which very little or no shrimping occurred in previous, more "normal" seasons. Further, the tonnages of shrimp caught were in quantities very nearly equal to "peak season" landings (see Table 1A and Figure 1A). Thus, Marine Extension Service researchers seized upon the opportunity and performed the study in December rather than waiting for the spring.

The Brunswick Harbor, the area in which this study was performed, was previously described by the Georgia Environmental Protection Division in 1976 (1):

> Brunswick Harbor (the portion of the East River where the State Docks are located) is very different from the rest of the Brunswick Estuary. Dikes built across both the East River and Academy Creek by the U.S. Army Corps of Engineers have essentially changed the Harbor into a tidal basin.

Further, this same study reported that the Brunswick Harbor was polluted by organic solids deposits from seafood related industrial discharges, urban runoff, sewer overflow, and from the Brunswick Wastewater

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Treatment Facility before Academy Creek was diverted from the Harbor.

However, even though the flow of fresh water into the East River has been effectively ceased by the placement of the aforementioned dikes, thus transforming it from a riverine system into a body of water influenced solely by tidal action, it nonetheless maintains significant BOD_5 and DO levels. Concurrent monitoring, sponsored by the Georgia Department of Natural Resources, Environmental Protection Division, performed by Dr. Eugene P. Keferl and fellow researchers at Brunswick Junior College (unpublished manuscript), enumerated the following annual averages: 2.2 mg/l BOD_5 and 6.2 mg/l DO (or 82% of saturation). More specifically, samplings performed on 12/07/77 by the Brunswick Junior College research team, one day after the first set of trawls and bottom grabs were performed by Marine Extension Service personnel in the same area, provided the following data: 2.1 mg/l BOD_5 and 6.7 mg/l DO (or 79% of saturation) (see Table 2A). Dr. Keferl's data suggest that the East River is not, in fact, "polluted", as described by the previous study (1).

MATERIALS AND METHODS

Two trawl areas were outlined for experimental sampling (see Figure 2A): Area A, extending from the Gulf Oil Company facility (at the base of London St.) to the north edge of the State Docks; and Area B, extending from the north edge of the State Docks to the South edge of the State Docks. Included within Trawl Area A was that portion of the East River directly in front of Packing Houses A, B, and C. The depth of the East River in Area A averages 15-17 ft. Trawl Area B, in front of the State Docks, has been dredged to an average depth of 28-30 ft. to facilitate the navigation and docking of larger, ocean-going ships. Figure 3A illustrates that at the north edge of the State Docks (the dividing line between Trawl Areas A and B), there occurs a sharp dropoff of the river bottom created by dredging activities.

All drags performed during the course of this study were accomplished through the use of a standard 40' shrimp trawl. Each drag was made along the center line of channel (see Figure 2A), and the duration of each drag was approximately 10 min. All bottom grabs were made with a 55 lb. steel grab sampler which had an 8" x 9" opening. Whenever shrimp heads were found, either by the dragging or bottom grab methods, the location was noted, the number of shrimp heads recorded, and the heads then returned to the location from which they were retrieved in order that they not be prematurely removed from the experiment. Likewise, all living marine organisms dragged up during the trawling efforts

were returned to the East River immediately after the searches for shrimp heads were completed.

On 12/06/77, initial samplings of the East River were performed by Marine Extension Service personnel. On this date, four trawls and eight bottom grabs were made. As listed in Table 1A, Packing Houses A and C were not operating from 12/01/77 through 12/06/77. However, Packing House B was discharging an average of 300 lb of shrimp heads per day during the same period, an amount which very nearly approximated the shrimp head discharge volumes generated during the height of the season. Noting these conditions, the Marine Extension Service prepared for the next phase of the study.

Table 1A illustrates that all three packing houses had begun operating one week later, on 12/13/77. On 12/14/77, 1104 lb of shrimp heads were collected from Packing House A (which does not normally discharge shrimp heads into the East River). During high slack tide (1030 - 1100), the shrimp heads were then dumped from the docks of Packing Houses A, B, and C, thus adding the following quantities of shrimp heads to the amounts already discharged by the three packing houses under normal operating conditions:

Packing House A   350 lb
Packing House B   416 lb
Packing House C   338 lb

The shrimp heads were dumped from the three docks during high slack tide because the local shrimpers and shrimp packers contended that the heads wash away from the docks, and are eventually flushed from the bay.

Table 3A summarizes the amounts of shrimp handled by Packing Houses A, B, and C from 12/06/77 through 12/19/77, the date of the last experimental samplings made by the Marine Extension Service. Likewise, the combined amounts of shrimp heads discharged by the three packing houses and the Marine Extension Service during the same period are listed. The total amount of shrimp handled at Packing Houses A, B, and C from 12/06/77 through 12/19/77 was 31,887 lb. The total amount of shrimp heads discharged at Packing Houses A, B, and C from 12/06/77 through 12/19/77, either as normal operating discharges by the packing houses them-selves, or as experimental discharges made by the Marine Extension Service, amounted to 8,515 lb.

Thus, trawls and bottom grabs were performed according to the following schedules:

Trawls

Date	Total Number of Trawls	Trawl Area A	Trawl Area B
12/06/77	4	3	1
12/14/77	2	1	1
12/15/77	2	1	1
Totals	8	5	3

Bottom Grabs

Date	Total Number of Grabs	At Dock	Out From Dock
12/06/77	8	-0-	8
12/14/77	21	13	8
12/15/77	9	9	-0-
12/16/77	18	8	10
12/19/77	16	9	7
Totals	72	39	33

Of the total 72 bottom grabs performed, 33 were made from either the R/V Capt. Gene or the smaller UGA runabout at distances of from 10 - 125 ft out from the packing houses' docks. The 39 dockside bottom grabs were made at three different locations at each packing house dock: (i) directly in front of the point of shrimp head discharge; (ii) upriver from the point of shrimp head discharge; and, (iii) downriver from the point of shrimp head discharge.

RESULTS AND DISCUSSION

As stated previously, 1104 lb of shrimp heads were dumped off the docks of Packing Houses A, B, and C on 12/14/77 at high slack tide because of the popular local contention that the shrimp heads are flushed from the bay by tidal action and/or are eaten by marine organisms such as catfish, turtles, eels, ducks, crabs, or other shrimp. Further, shrimpers and shrimp packing house operators contend that prop wash created by the shrimp boats entering and leaving the packing houses' dock areas is sufficient in magnitude to churn the shrimp heads from the river bottom, thus allowing them to be

more readily flushed from the area.

Table 1 illustrates that of the total of eight trawls performed on 12/06/77, 12/14/77, and 12/15/77, only three produced shrimp heads (or fragments therefrom). Shrimp heads were dragged up during one trawl of Area A and two trawls of Area B. The relatively small number of shrimp heads (1 - 6 heads per trawl) found by dragging the center line of channel indicate that the shrimp heads, once washed away from the docks, do not remain in the bay.

Although six shrimp fragments were recovered in one drag of Area B on 12/06/77 (see Table 1), Table 2 illustrates that no shrimp heads were found in the eight bottom grabs made at various distances away from the packing houses' docks on the same date. Furthermore, after the experimental dumping of shrimp heads from the three packing houses' docks on 12/14/77, no shrimp heads were found in bottom grabs made at any distance away from the docks, with the exception of one shrimp antenna found in a grab sample taken 50 ft off of the Packing House B dock on 12/19/77 (see Tables 2, 3, 4, 5, and 6).

Large amounts of shrimp heads were found, however, at the points of discharge of heads during and up to two days after heading at Packing Houses B and C. Similarly, large amounts of shrimp heads were found at the points where the previously-described experimental lots were dumped off of the docks of Packing Houses A, B, and C, and continued to be found for up to three days after they were dumped under experimental conditions. However, grab samples taken on 12/19/77 (Table 6) revealed that all or almost all shrimp heads were no longer present in front of the three packing houses' docks. For the purposes of discussion, each packing house dock will be considered individually.

The point at which 350 lb of shrimp heads were dumped at Packing House A on 12/14/77 was monitored closely through the use of the grab sampler. On 12/14/77, large amounts of heads were collected at the dumping point (see Table 3). Similarly, large amounts were collected on 12/15/77 and 12/16/77 (see Tables 4 and 5). However, on 12/19/77, only four shrimp heads were found in one grab out of four made at Dock A (see Table 6). These results illustrate that large quantities of shrimp heads were washed away from the dock at Packing House A within three days.

Besides the 416 lb of shrimp heads dumped at Packing House B on 12/14/77 by the Marine Extension Service, an additional 500 - 1000 lb of shrimp heads were seen on the bank beneath the dock at low water. From 12/14/77 through 12/16/77, large amounts of shrimp heads were collected in bottom grabs taken from the edge of the dock (see Tables 3, 4, and 5). However, on 12/19/77, only three heads were found at the same

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edge-of-dock point, illustrating that large amounts of shrimp heads were also washed away from the dock at Packing House B within three days' time (see Table 6).

At Packing House C, the same conditions were found to exist. Although as many as 300 - 400 shrimp heads could be collected per grab on 12/16/77 (see Table 5), samples taken on 12/19/77 revealed only three shrimp heads in one grab out of three (see Table 6).

CONCLUSIONS

Thus, utilizing a conversion factor of 85 shrimp heads/lb (even though 8,515 lb of shrimp heads were discharged at the docks of Packing Houses A, B, and C from 12/06/77 through 12/19/77) totals of only 0.1 and 14.8 lb of shrimp heads were recoverable by eight trawls and 72 bottom grabs, respectively. Therefore, it seems apparent that large quantities of shrimp heads, as normally discharged by the three packing houses, are indeed washed away from the docks. Further, it may be stated that the period of time required for adequate flushing appears to be 2 - 4 days.

TABLE 1. Experimental Trawls.

Date	Trawl	Trawl Location*	Number of Shrimp Fragments	Other (Specify)
12/06/77	01	A	-0-	Fish**, shrimp, crab, mud, wood chips, log.
12/06/77	02	A	-0-	Fish, shrimp, crab, log, crab trap.
12/06/77	03	A	-0-	Fish, shrimp, crab.
12/06/77	04	B	6	Fish, shrimp, crab.
12/14/77	05	B	1	Fish, shrimp, crab.
12/14/77	06	A	-0-	Fish, shrimp, crab.
12/15/77	07	B	-0-	Fish, shrimp, crab.
12/15/77	08	A	1	Fish, shrimp, crab.

*Trawl Location A: East River (Oglethorpe Bay) from Gulf Oil dock to north edge of the State Docks.
 Trawl Location B: East River (Oglethorpe Bay) from north edge of State Docks to south edge of State Docks.

**A representative trawl included: 1 blue, 3 flounder, 3 pike, 4 drum, 24 spot, and 36 summer trout.

TABLE 2. Bottom Grabs, 12/06/77.

Time	Sampling Location Dock	At Dock	Out From Dock	Feet From Dock	No. Shrimp Heads	Other (Specify)
NA	A		d*	60	-0-	Crab shell fragments, peri-winkle, marsh grass, rocks, mud.
NA	A		d	100	-0-	Mud.
NA	B		d	60	-0-	Mud.
NA	B		d	100	-0-	Mud.
NA	C		d	60	-0-	Mud.
NA	C		d	100	-0-	Mud.
NA	SD**		d	125	-0-	---
NA	SD		d	125	-0-	---

(Grab No.: 01, 02, 03, 04, 05, 06, 07, 08)

*d = Grab sample taken downriver from point of shrimp head discharge.

**SD = Grab samples taken in front of State Docks, one at north edge, one at south edge, approximately at center line of channel.

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TABLE 3. Bottom Grabs, 12/14/77.

		Sampling Location					
Time	Grab No.	Dock	At Dock	Out From Dock	Feet From Dock	No. Shrimp Heads	Other (Specify)
1330	01	A		d*	30	-0-	Crab claws.
1345	02	A		d	20	-0-	Crab claws.
1350	03	A		d	10	-0-	Crab claws, oyster shells.
1355	04	B		d	10	-0-	Oyster shells.
1400	05	B		d	20	-0-	Oyster shells.
1405	06	B		d	30	-0-	Oyster shells.
1408	07	C		d	10	-0-	Axe blade.
1410	08	C	u*			-0-	Oyster shells.
1415	09	C	f*			-0-	Chain, oyster shell.
1418	10	C		d	20	-0-	Oyster shells, 1 small, dead trash fish.
1615	11	C	u			-0-	Oyster shells.

*d = Grab sample taken downriver from point of shrimp head discharge.
 u = Grab sample taken upriver from point of shrimp head discharge.
 f = Grab sample taken in front of point of shrimp head discharge.

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TABLE 3 (continued). Bottom Grabs, 12/14/77.

| | | Sampling Location | | | | |
Time	Grab No.	Dock	At Dock	Out From Dock	Feet From Dock	No. Shrimp Heads	Other (Specify)
1617	12	C	f*			-0-	Brick.
1619	13	C	d*			-0-	Mud.
1621	14	C	d			-0-	Mud.
1623	15	C	f			50	—
1625	16	B	u*			-0-	Mud.
1630	17	B	f			-0-	Mud.
1633	18	B	d			-0-	Mud.
1635	19	A	u			-0-	Conch shells.
1640	20	A	f			12	Crab waste.
1643	21	A	d			-0-	Crab waste.

*d = Grab sample taken downriver from point of shrimp head discharge.
 u = Grab sample taken upriver from point of shrimp head discharge.
 f = Grab sample taken in front of point of shrimp head discharge.

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TABLE 4. Bottom Grabs, 12/15/77.

| | | Sampling Location | | | | No. | |
Time	Grab No.	Dock	At Dock	Out From Dock	Feet From Dock	Shrimp Heads	Other (Specify)
0905	01	C	d*			-0-	Oyster shells.
0910	02	C	f*			142	--
0915	03	C	u*			-0-	Brick.
0920	04	B	d			40	--
0925	05	B	f			287	--
0930	06	B	u			1	--
0945	07	A	f			160	--
0950	08	A	d			1	Crab waste.
0955	09	A	d			-0-	Crab waste.

*d = Grab sample taken downriver from point of shrimp head discharge.
u = Grab sample taken upriver from point of shrimp head discharge.
f = Grab sample taken in front of point of shrimp head discharge.

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TABLE 5. Bottom Grabs, 12/16/77.

| | | Sampling Location | | | | | |
| | | | | | No. | | |
Time	Grab No.	Dock	At Dock	Out From Dock	Feet From Dock	Shrimp Heads	Other (Specify)
0635	01	A		d*	20	-0-	Mud.
0637	02	A		d	40	-0-	Mud.
0640	03	A		d	100	-0-	Mud.
0645	04	B		d	30	-0-	Mud.
0648	05	B		d	50	-0-	Mud.
0650	06	B		d	100	-0-	Mud.
0700	07	B		d	25	-0-	Mud, oyster shells.
0705	08	C		d	30	-0-	Mud, oyster shells.
0710	09	C		d	60	-0-	Mud.
0712	10	C		d	100	-0-	Mud.
0930	11	C	u*			-0-	Oyster shells.
0935	12	C	f*			350	---

*d = Grab sample taken downriver from point of shrimp head discharge.
 u = Grab sample taken upriver from point of shrimp head discharge.
 f = Grab sample taken in front of point of shrimp head discharge.

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TABLE 5 (continued). Bottom Grabs, 12/16/77.

Time	Grab No.	Sampling Location					No. Shrimp Heads	Other (Specify)
		Dock	At Dock	Out From Dock	Feet From Dock			
0942	13	B	u*				-0-	Oyster shells.
0945	14	B	f*				3	Mud.
0950	15	B	d*				-0-	Mud, rock.
1000	16	A	d				-0-	Mud.
1003	17	A	f				1	Crab waste.
1007	18	A	f				200	Crab waste.

*d = Grab sample taken downriver from point of shrimp head discharge.
 u = Grab sample taken upriver form point of shrimp head discharge.
 f = Grab sample taken in front of point of shrimp head discharge.

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TABLE 6. Bottom Grabs, 12/19/77.

Time	Grab No.	Sampling Location				No. Shrimp Heads	Other (Specify)
		Dock	At Dock	Out From Dock	Feet From Dock		
1512	01	A	d*			-0-	Conch, crab waste.
1514	02	A	d			-0-	Conch, crab waste.
1515	03	A	f*			4	Crab waste.
1517	04	A	f			-0-	Conch, crab waste.
1520	05	A		d	15	-0-	Conch shells, brick.
1525	06	A		d	50	-0-	Mud.
1528	07	A		d	15	-0-	Clam shells, oyster shells.
1532	08	A		d	25	-0-	Oyster shells.
1538	09	B	f			3	Mud.
1541	10	B	f			-0-	Mud, oyster shells.
1544	11	B		d	30	-0-	---
1550	12	B		d	50	1**	Mud, conch shell.

*d = Grab sample taken downriver from point of shrimp head discharge.
 u = Grab sample taken upriver from point of shrimp head discharge.
 f = Grab sample taken in front of point of shrimp head discharge.

**Shrimp antenna only.

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TABLE 6 (continued). Bottom Grabs, 12/19/77.

Time	Grab No.	Sampling Location				No. Shrimp Heads	Other (Specify)
		Dock	At Dock	Out From Dock	Feet From Dock		
1555	13	C	f*			3	---
1557	14	C	f			-0-	Oyster shells.
1600	15	C	f			-0-	Oyster shells.
1605	16	C		d*	50	-0-	Mud.

*d = Grab sample taken downriver from point of shrimp head discharge.
 u = Grab sample taken upriver from point of shrimp head discharge.
 f = Grab sample taken in front of point of shrimp head discharge.

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ACKNOWLEDGEMENTS

REFERENCE

1. GEORGIA ENVIRONMENTAL PROTECTION DIVISION. August, 1976. Brunswick Estuary Study. Georgia Department of Natural Resources. p. 1.

APPENDIX

(Flushing of Shrimp Heads from Coastal Waters)

TABLE 1A. East River Packing Houses Data, December, 1977 (Pounds).

Date	Dock A		Dock B		Dock C	
	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock
12/01/77	-0-	-0-	1121	393	-0-	-0-
12/02/77	-0-	-0-	1019	357	-0-	-0-
12/03/77	-0-	-0-	825	289	-0-	-0-
12/04/77	-0-	-0-	203	71	-0-	-0-
12/05/77	-0-	-0-	314	110	-0-	-0-
12/06/77	-0-	-0-	1649	578	-0-	-0-
12/07/77	-0-	-0-	548	192	-0-	-0-
12/08/77	933	-0-	265	93	-0-	-0-
12/09/77	-0-	-0-	467	164	-0-	-0-
12/10/77	-0-	-0-	3237	1135	-0-	-0-
12/11/77	-0-	-0-	2424	850	-0-	-0-
12/12/77	1697	-0-	464	163	-0-	-0-
12/13/77	-0-	-0-	619	217	1047	367
12/14/77	1434	-0-	1244	436	2974	1042

TABLE 1A (continued). East River Packing Houses Data, December, 1977 (Pounds).

Date	Dock A		Dock B		Dock C	
	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock
12/15/77	2902	-0-	1431	502	1637	574
12/16/77	2656	-0-	846	297	-0-	-0-
12/17/77	265	-0-	279	98	-0-	-0-
12/18/77	-0-	-0-	527	185	-0-	-0-
12/19/77	865	-0-	442	155	1035	363
12/20/77	982	-0-	482	169	1035	363
12/21/77	-0-	-0-	787	276	122	43
12/22/77	-0-	-0-	263	92	-0-	-0-
12/23/77	246	-0-	845	296	-0-	-0-
12/24/77	-0-	-0-	-0-	-0-	-0-	-0-
12/25/77	-0-	-0-	-0-	-0-	-0-	-0-
12/26/77	-0-	-0-	-0-	-0-	-0-	-0-
12/27/77	661	-0-	400	140	-0-	-0-
12/28/77	1155	-0-	2476	868	1137	399

TABLE 1A (continued). East River Packing Houses Data, December, 1977 (Pounds).

Date	Dock A		Dock B		Dock C	
	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock
12/29/77	476	-0-	1361	477	8662	3037
12/30/77	1400	-0-	476	167	1910	670
12/31/77	895	-0-	554	194	-0-	-0-
Totals	16567	-0-	25568	8964	19559	6858

TABLE 2A. Brunswick Harbor BOD$_5$ and DO, 1977*.

Sampling Date	BOD$_5$ (mg/l)	DO (mg/l)	Saturated DO (mg/l)	DO (% of Saturation)
02/16/77	1.9	9.4	10.1	93
03/16/77	1.2	6.0	8.9	67
04/13/77	2.4	7.9	8.0	99
05/18/77	2.5	8.2	8.0	103
06/15/77	1.8	6.9	6.7	103
07/13/77	--	6.4	6.3	102
08/10/77	4.2	4.8	6.2	77
09/14/77	1.7	3.6	6.6	55
10/12/77	--	4.2	7.2	58
11/09/77	2.0	4.7	7.7	61
12/07/77	2.1	6.7	8.5	79

*From Dr. Eugene P. Keferl, unpublished manuscript.

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TABLE 3A. East River Packing Houses Data During Experimental Period (Pounds).

Period	Dock A		Dock B		Dock C	
	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock	Shrimp Handled At Dock (Heads On)	Shrimp Heads Discharged At Dock
12/06/77 thru 12/19/77	10752	-0-	14442	5065	6693	2346
Experimental Dischges (12/14/77)	-0-	350	-0-	416	-0-	338
Totals	10752	350	14442	5481	6693	2684

Total Shrimp Handled at Docks A, B, and C from 12/06/77 thru 12/19/77   31887

Total Shrimp Heads Discharged at Docks A, B, and C from 12/06/77 thru 12/19/77   8515
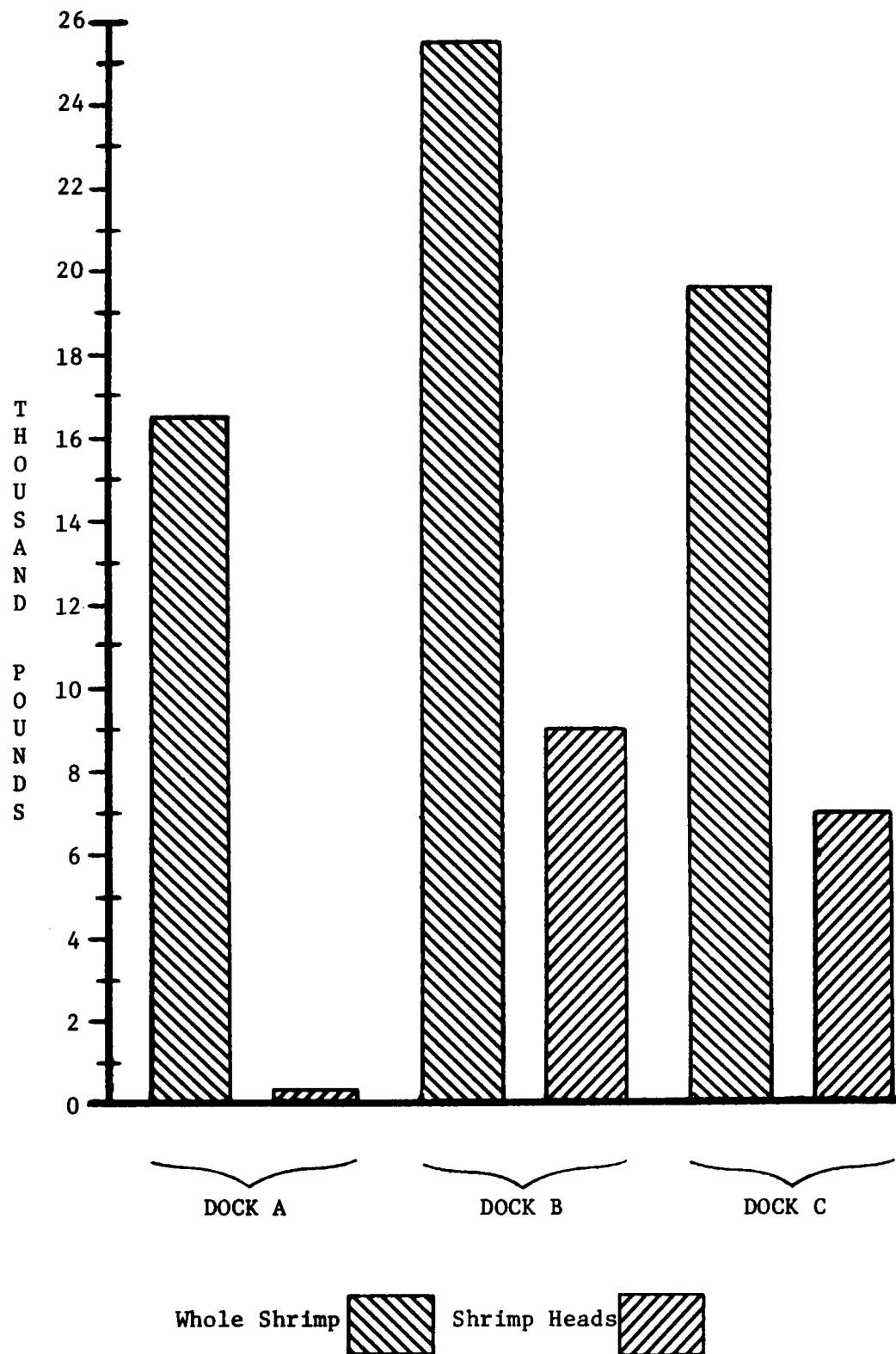
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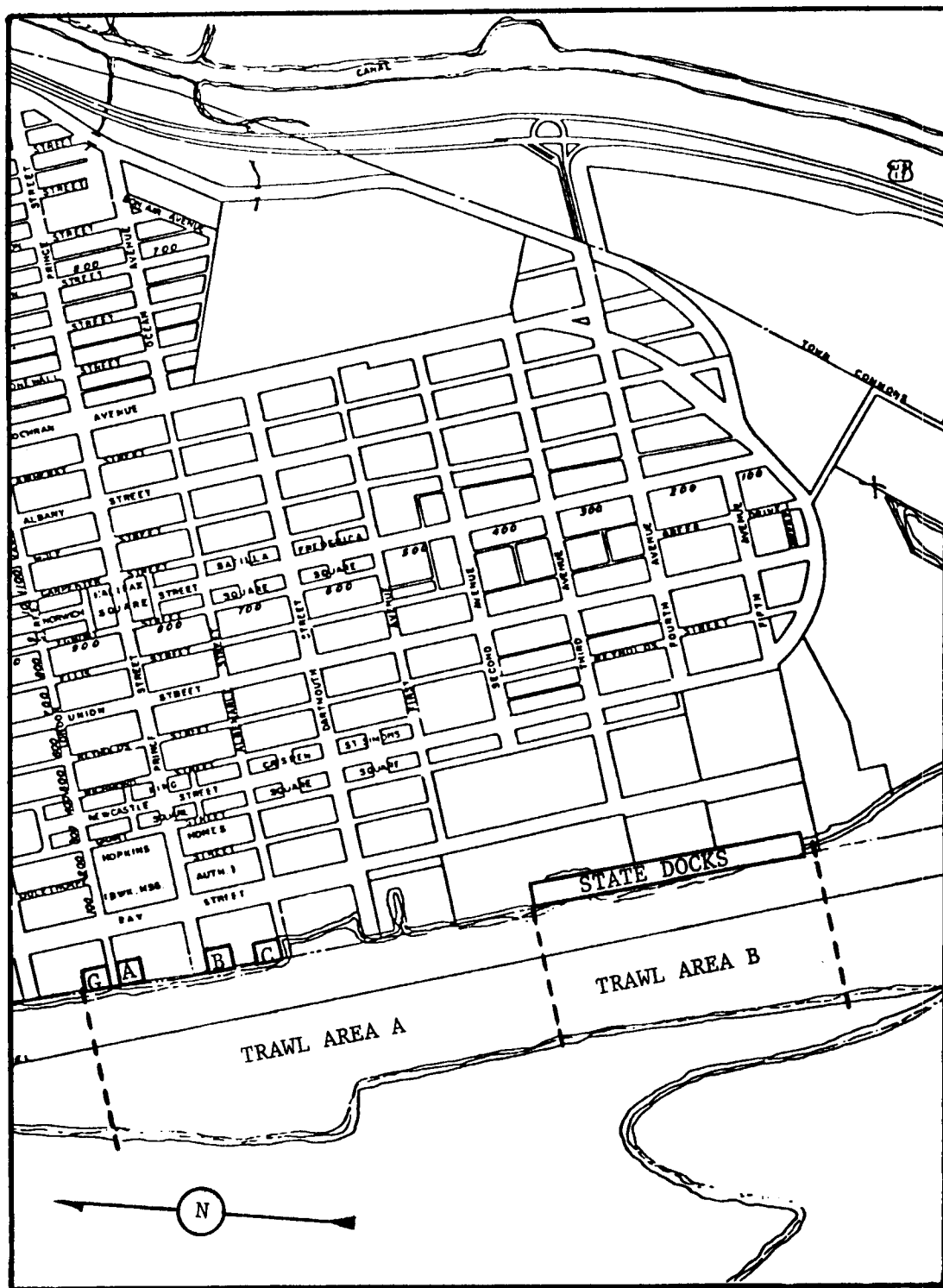FIGURE 1A. East River Packing Houses' Data, December, 1977.

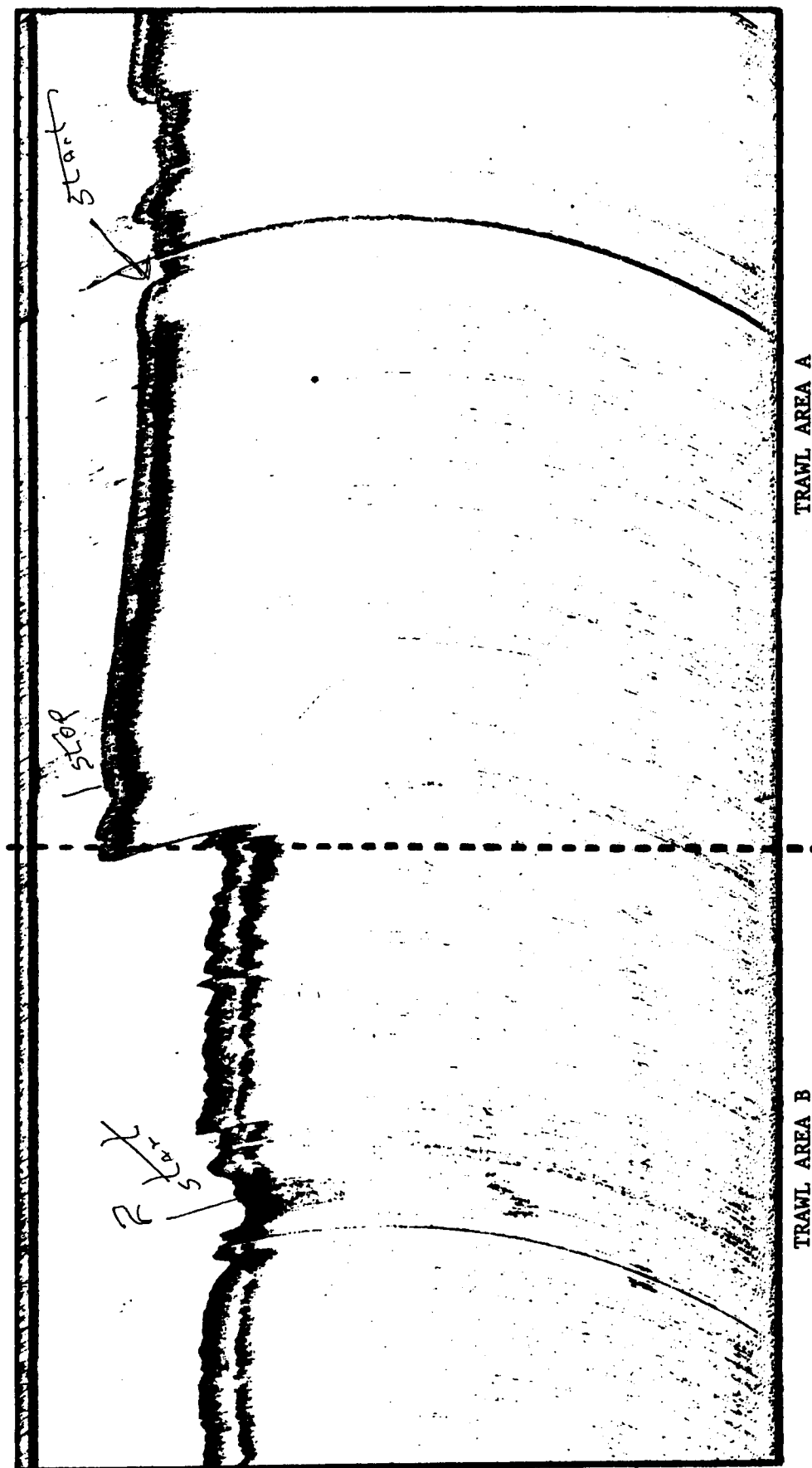FIGURE 2A. East River Trawl and Bottom Grab Location Map.

FIGURE 3A. Fathometer Chart of East River Bottom*.

*(Chart from SIMRAD Echosounder Model EL.)

TRAWL AREA A

TRAWL AREA B

DRY CLEAN-UP TECHNIQUES FOR REDUCING THE
BOD WASTE LOAD FROM SHRIMP PROCESSORS

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One of the major seafood technology projects of the University of Georgia Marine Extension Service in 1976-1977 was the reduction of waste loads from a shrimp processing plant. The plant requesting assistance processed 8 to 10 tons per day of green headless shrimp while producing several breaded shrimp products. As with other food industries, wastewaters from a breaded shrimp plant are typically 10-fold more concentrated than domestic sewage. In order to obtain a discharge permit under the NPDES system being administered by the State of Georgia, the plant needed to reduce its pollution load by 80% corresponding to the equivalent of secondary treatment. The plant was located on a "water quality limited segment" of the estuary and treatment more stringent than the effluent limitation guidelines set by the U. S. Environmental Protection Agency, for Best Practicable Control Technology, was required. The alternative solution for this plant would have been to discharge its wastewaters to the municipal treatment system or build a treatment system of its own. Another alternative was to reduce the pollution load in-plant by dry clean-up practices and by-product recovery. These measures, combined with the reduction achieved by a hydro-sieve screen previously installed, were effective for removing approximately 70% of the waste load measured as Biochemical Oxygen Demand (BOD). The BOD test determines the amount of dissolved oxygen required by micro-organisms for aerobic decomposition of organic matter present in water. In this case, the organic matter comes from seafood processing wastes which contain small particles of shrimp flesh, breading, soluble proteins, and carbohydrates. Plans were made to further reduce the remaining waste load by segregating effluents from the breading operation and discharging these wastewaters (approximately 10% of total BOD load) to the municipal treatment system.

This bulletin outlines the recommendations made and their effect on reducing the waste load of BOD from a breaded shrimp plant. These recommendations would be applicable in other seafood plants and for dry clean-up and by-product recovery practices throughout the food processing industry.

MATERIALS AND METHODS

Being an advisory project rather than a research study, the first element of the project was a meeting with top management of the seafood company to discuss their problems in obtaining a permit to discharge wastewater. Knowing from experience that most food plants can reduce processing and clean-up waste loadings by 25-50% prompted me to suggest an inspection of the plant with a report to management. The suggestion was taken and the first inspection occured the same night. Subsequent inspections and demonstrations of dry clean-up practices used such commonly available materials as boots, brooms, shovels, pans, bags, barrels, and squeeges to accomplish the objective of reducing the waste load. Obviously, some retraining of the clean-up crew and rethinking by top management preceeded this wielding of brooms and shovels prior to wash down.

Accurate sampling of the wastewater to assess the effects of our dry clean-up recommendations required frequent sampling on a 24 hour basis. During processing, grab samples and flow rates were taken every 30 minutes and then composited in proportion to flow. Clean-up periods during the day and the main event at night were sampled every 15 minutes and composited in proportion to flow. Laboratory analyses were conducted by our research technicians working together with quality control and engineering personnel from the seafood plant. Analyses for BOD were made according to standard methods (1).

RESULTS AND DISCUSSION

A number of concentrated liquid wastes were generated in the plant. Thick viscous batter dripped from tables in the breading department. Prior to catching this material in pans as recommended, this material was allowed to enter the floor drains. During clean-up, the thick batter on the floor, now squeeged into pans, was previously washed down the drain. Also batter tanks which had previously been pumped to the floor drain, are now emptied into barrels.

Dry batter that falls from tables, machines, or product conveyor belts is a concentrated source of soluble and suspended BOD. Trays can be placed under machines to catch breading which would otherwise fall onto the floor. Clean-up of machines such as sifters and tables would certainly be most easily accomplished by blasting the leftover breading off with an air gun or water hose, but the

effort expended in removing it by hand was necessary to reduce the waste load. It was found in the course of this study that each pound of dry breading material contributed 0.4 pound of BOD to the soluble waste load. This proves the absolute necessity of keeping breading off the floor and out of the drains.

Sanitation is still an important concern when using dry clean-up practices. Brushes, squeeges, and scrapers which come in contact with product tables should not also be used on the floors. Separate storage lockers should be assigned for this equipment to keep it apart from regular wet clean-up gear. Bending and scraping is the only way to recover breading from under equipment and tables. Any breading material that falls to the floor must be swept up with a stiff broom, collected in a pan or shovel, and placed into barrels or bags. Ultimate disposition of the breading materials from the plant is usually as simple as giving or selling it to hog farms in the area. Such materials are a desirable source of carbohydrates and energy in animal rations.

Shrimp hulls can be a source of soluble BOD and contribute to the waste load. Previous studies by Stephens et al. (5) have shown that shrimp heads and hulls contain enzymes which will degrade proteins adhering to the hull. This action causes a solid waste such as hulls to be partially solubilized producing a protein or peptone soup which contributes to the soluble BOD load. Thus, the hulls can be a source of pollution when left in contact with the water. Hulls should be removed by dry clean-up practices without water where possible.

Unavoidably some shrimp hulls will enter the floor drains, along with large particles of breading, and other particulate wastes. These particulate wastes can be effectively removed by screens, such as the commonly used hydro-sieve screen. Particles of solid wastes removed by the screen can then be collected in a dumpster and buried in an approved land fill or rendered into a meal for animal feed.

The screening system substantially reduced the BOD load from processing and clean-up as shown in Table 1 even before the dry clean-up practices were instituted. The numbers are expressed as pounds BOD per 1000 pounds of green headless shrimp processed. In the situation considered, the processing BOD load was reduced from 117 pounds to 72 pounds by screening. The clean-up BOD load was reduced from 104 pounds to 49 pounds by the hydro-sieve screening system which removed particles larger than 0.02 inches in diameter. The total BOD load on a daily basis was reduced from 221 to 121 pounds. Thus, the BOD load was reduced to 55% of the total raw waste load by screening.

Further reductions in the BOD load were made by adopting the dry clean-up practices previously discussed. The dry clean-up practices resulted in essentially no

difference in the BOD load from processing. This load was 72 pounds after screening compared to 71 pounds after screening and implementation of dry clean-up practices. The anticipated reduction in BOD load was obtained by using dry clean-up prior to washdown. The clean-up load after screening (49 pounds BOD per 1000 pounds shrimp processed) was reduced to 21 pounds BOD per 1000 pound shrimp processed by adopting the dry clean-up practices This clean-up load was reduced by 57% and the total plant load was reduced by 24% from 121 pounds after screening to 92 pounds by implementing the dry clean-up practices.

These results are graphically summarized in Figure 1. The total raw waste load determined in this study is shown in Column C as 100%. This corresponds to 4658 pounds BOD per day, and is not based on 1000 pounds green headless as was the previous data. The hydro-sieve screen reduced the waste load to 55% of the total raw load by removing particles larger that 0.02 inches in diameter. This is shown in Column D and corresponds to a BOD load of 2561 pounds per day. The effect of dry clean-up and by-product recovery reduced the waste load to 1462 pounds BOD per day (Column E) or 31% of the total raw waste load before screening.

The data shown in Columns A & B illustrates the problems of determing the total raw waste discharge from the plant. In this study, the shrimp processing plant was required to reduce its waste laod by 80% in order to obtain an NPDES permit. Thus, it was obviously important to determine the amount of the total raw waste load prior to any treatment. Column A (1879 pounds of BOD) was the commonly accepted waste load of this plant, based upon an earlier published report by Horne (3). This earlier data was based on the average of five days sampling using samples taken hourly over 4-8 hours of operation. Another data point given in Figure 1, Column B, which correlates to 6356 pounds BOD per day was reported in the earlier published study but not included in the average because the sample was observed to be "high in meal from breading department". At the initiation of this present study of the processing plant, observation of the clean-up operation revealed that the majority of the clean-up load was being washed down the drain soon after the water hoses were turned on. Thus, very frequent sample intervals (15 minutes) were necessary to accurately assess the true load from clean-up.

CONCLUSION

The screening system removed nearly half of the total raw wasteload of BOD. This was further reduced to 31% of the total load by recovering by-products such as breading and batter and by using dry clean-up practices prior to turning on the hoses for washdown. Thus, nearly 70% of the total raw waste load was removed by screening and

good housekeeping practices, which, except for dry clean-up was in essence the technology recommended by the Federal Environmental Protection Agency to achieve the July 1, 1977 Effluent Limitation Guidelines for Breaded Shrimp Processing in the Contiguous State Subcategory. As stated in the Federal Register of June 26, 1974, p. 23139, "the best practicable control technology currently available consists of solids or by-product recovery through the use of screening systems and "good housekeeping" practices which are considered normal practice within the seafood processing industry such as turning off faucets and hoses when not in use or using spring loaded hose nozzles."

Having removed 70% of the BOD load by screening and in-plant measures, another 10% of the total raw waste load of BOD had to be removed to satisfy state water quality limitations of the proposed receiving waters. Plans were made to segregate wastewater from the breading room and from a high pressure cooking operation and discharge these wastes to the municipal treatment system which already receives the domestic sewage from the plant.

This approach to waste treatment involved production and clean-up personnel and required the commitment of top management. This was certainly more trouble and more expensive than conventional wet clean-up, but the savings in waste treatment costs made the reward even more worthwhile. The engineering staff of the shrimp processing plant calculated a savings of approximately 90% in capital construction costs by removing wastes in-plant rather than providing end-of-pipe removal for all of the BOD load. Municipal treatment for all of the initial waste load would have meant construction of bigger sewer lines, costsharing in proportion to their waste load for capital, operating, and replacement costs. In short, a comparison of municipal treatment vs. construction of their own waste treatment system revealed the fact that they were about equally expensive. Cost figures for treating wastewater from a plant this size were given in an earlier Marine Extension Bulletin by Smith and Bough (4).

If these recommendations for dry clean-up and by-product recovery are of interest to you, copies of a Marine Extension Bulletin (2) are availabe which includes a fold-out chart illustrating the techniques employed in this study.

ACKNOWLEDGEMENTS

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5. STEPHENS, N.L., and W. A. BOUGH, L.R. BEUCHAT, AND E.K. HEATON, 1976. Preparation and evaluation of two microbiological media from shrimp heads and hulls. Appl. and Environ. Microbiol. 31: 1-6.

TABLE 1

SCREENING AND BOD LOADS/1000 lb.
G. H. PROCESSED

OPERATION	BEFORE SCREEN	AFTER SCREEN
PROCESSING	117	72
CLEAN-UP	104	49
TOTAL	221	121

TABLE 2

DRY CLEAN-UP AND SCREENING ON
BOD LOADS/1000 lb G. H. PROCESSED

	AFTER SCREEN	DRY CLEAN-UP
PROCESSING	72	71
CLEAN-UP	49	21
TOTAL	121	92

A. Horne,1972,Aug.,1879 lb

B. Horne,1972,April,6356 lb

C. Before Screen, 4658 lb

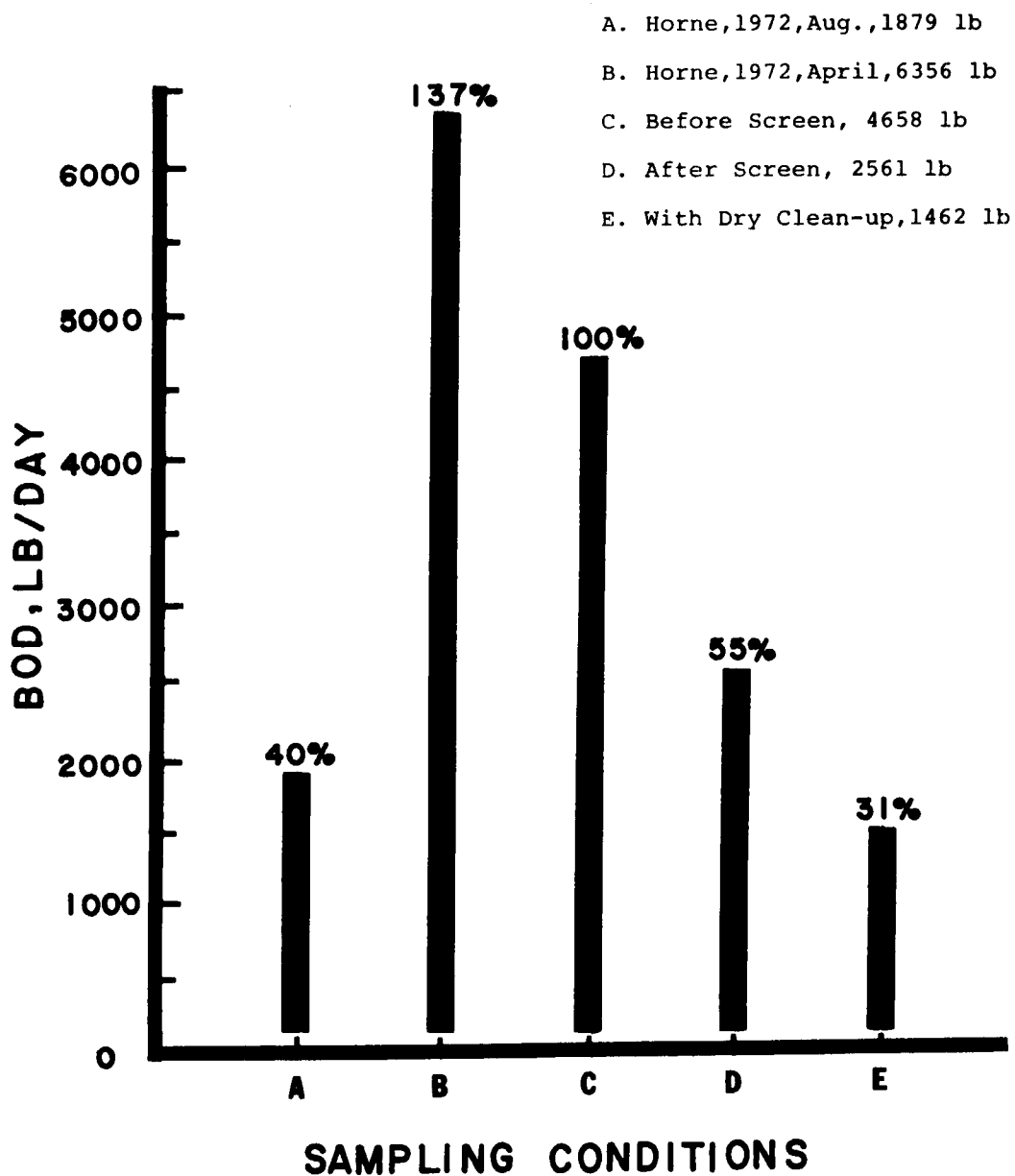D. After Screen, 2561 lb

E. With Dry Clean-up,1462 lb

Figure 1. Reduction Of Total BOD Loads From A Breaded Shrimp Processing Plant By Screening And Dry Clean-Up

CHARACTERIZATION OF WASTE LOADING FROM A SHRIMP PACKING HOUSE AND EVALUATION OF DIFFERENT SCREEN SIZES FOR REMOVAL OF HEADS

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Environmental quality of Georgia's coastal waters is of growing concern. The screening requirements applied by the Environmental Protection Division (EPD) of Georgia to wastewater from shrimp packing houses is also of growing concern. Prior to this study, EPD requirements called for screening of packing house wastewater through at least a 40 mesh screen. (6) Preliminary discussions with the EPD revealed the main function of the screen was to remove shrimp heads. It was postulated that larger screen sizes would remove heads (approximately 1.5 x 0.5 inch) and not be as likely to clog, bind, or split as a 40 mesh screen. Thus, the offer by the Marine Extension Service to conduct research studies on screening requirements was accepted by EPD. We were to determine waste loadings of Biochemical Oxygen Demand (BOD) and Suspended Solids (SS), and we were to assess the effectiveness of BOD and SS removal by three different screen sizes (1/16, 1/4, and 1/2 inch openings) from the effluent generated by a shrimp packing house. Two different heading techniques, commonly known as "dry" heading and "wet" heading, were to be evaluated in the studies. The information would be used to assist shrimp packing houses and provide the Georgia EPD with an analytical base upon which to base their screening regulations.

In May of 1977, the Director of the Georgia EPD delayed the screening requirement for one year while studies were made by the Seafood Technology Laboratory of the University of Georgia Marine Extension Service. This report concerns our evaluation of different sizes to minimize detrimental effects of packing house effluent on the environmental quality of coastal waters and at the same time allow the use of a screen simple to build, easy to operate, and economically practical for the packing houses.

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MATERIALS AND METHODS

The study was conducted at a local shrimp packing facility located on Oglethorpe Bay (East River) in Brunswick, Georgia. Two different methods were tested in the heading of shrimp. They are commonly known as the "dry" heading technique and the "wet" heading technique. The major difference between the two techniques is whether the heads are kept "dry" by placing them in buckets or handled "wet" by fluming. Using the "dry" method, the head is placed in a container that will later be weighed for the payment of the person who headed the shrimp. The tail is flumed to the end of the table and caught in a basket typically with 3/8 x 4 inch openings. In the "wet" heading technique, the head is placed in the flume while the tail is placed in a container for weighing. Regardless of heading technique, the head is usually disposed of by one of the following means: (i) discharged into the bay; (ii) rendered into animal feed; or (iii) used as bait for catfish and eels. See Figures 1A and 2A for schematic representations of the "dry" and "wet" heading techniques.

When a boat arrives at the docks of a shrimp packing house with shrimp to be headed, they are unloaded and heads-on shrimp are brought to the heading table. Depending on how much shrimp a particular boat caught, the catch may be headed in a period of 30 minutes to 2 hours. Packing houses do not operate throughout the day, but only when shrimp boats arrive at the dock that have not headed their shrimp at sea. Boats with small catches will usually have headed their shrimp at sea in order to obtain a higher price at the dock. The number of workers (usually between 10 to 20 in Georgia) does not always vary in direct proportion to the amount of the catch. Obviously, the number of workers at a table heading a particular amount of shrimp will influence the strength of the wastewater. Similarly, if fewer workers take longer to head a particular catch of shrimp, the concentration may be less but the volume of wastewater and the processing period will be longer. To account for such variation, waste loadings were determined in pounds per day and the ratios calculated of BOD and suspended solids per thousand pounds of green headless (G.H.) shrimp packed. Such loading ratios also provide data that can be extrapolated to other packing houses or to the same packing house handling different quantities of shrimp.

Samples were taken of shrimp heading table effluents (from both "dry" and "wet" heading processes) discharging into a large rectangular tray, which then emptied through a four inch drain hole into Oglethorpe Bay. A plastic pipe with a collar was tightly fitted into the four inch drain hole. To the collar of the

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plastic pipe was attached a cylinder of screen having either 1/16, 1/4, or 1/2 inch square openings. All effluent passed through a wire basket having rectangular (3/8 x 4 inch) openings before passing through the screen being tested. In the "dry" heading technique, the tail is caught in the basket for transfer to the wash vat. In the "wet" heading technique, the head is caught in the basket for disposal.

Each sampling of the heading table effluent took place within a 15 minute period, with five 300 ml samples of the effluent taken before and after each screen being tested. During the 15 minute sampling period, the flow rate of the effluent was taken three times and then averaged to estimate the flow rate for each specific sampling period. At the end of the 15 minute period, the 1500 ml of effluent collected before and after the screen were composited into two separate 500 ml samples and stored on ice in a cooler until transported to the laboratory. A portion of each effluent sample was used for SS determination (1) before freezing. The remainder of the sample was frozen at -5°C within two hours after collection. Frozen samples were thawed out under running tap water for BOD determinations (2).

After the shrimp are headed, they are placed in a wash vat for further cleaning. Three 500 ml samples of the wash vat effluent samples were taken and composited into one 500 ml sample and handeled as above. The BOD and SS values for raw unscreened samples determined for the wash vat effluent samples were added to the values for BOD and SS for the heading table effluent samples, in order to calculate the total waste load of the effluent from the overall shrimp packing process without any screening. On each day the effluent was sampled, the total time of operation and pounds shrimp headed (G.H.) were noted and recorded.

Statistical analysis of the data contained in Tables 1, 2, and 3 were performed. The average waste load from the "dry" and "wet" heading technique in Tables 1 and 2 were tested for analysis of variance to determine if there was a significant difference between the waste loads of the two heading techniques. In Table 3, the percent reduction of each size screen was tested for significant reduction using the one-tailed t-test.

RESULTS AND DISCUSSION

The results from unscreened waste load (BOD and SS) determinations for the "dry" heading technique are contained in Table 1. The BOD load per day varied from 3.8 pounds (Sept. 20) to 21.5 pounds (Sept. 19), while the SS load varied from 0.6 pounds (Sept. 29) to 3.2 pounds (Sept. 19). During this period, the quantity of

shrimp headed varied from 392 pounds (Sept. 29) to 2,798 pounds (Sept. 19). However, the loading ratio (BOD and SS lbs/1000 lbs G.H.) showed less variability. The BOD and SS average of 24 samples collected on four different days were 9.1 ± 4.7 lbs/1000 lbs G.H. and 1.3 ± 0.2 lbs/1000 lbs G.H., respectively.

The results of the unscreened waste load determinations (BOD and SS) from the "wet" heading technique are contained in Table 2. The average BOD loading ratio (17.4 ± 4.4 lbs/1000 lbs G.H.) observed to be nearly twice as great for "wet" heading as for "dry" heading (9.1 ± 4.7 lbs/1000 lbs G.H.). Similarly, the average ratio of suspended solids (5.8 ± 1.5 lbs/1000 lbs G.H.) was approximately 4-fold greater for "wet" heading than for "dry" heading (1.3 ± 0.2 lbs/1000 lbs G.H.). These loading ratios represent nine samplings collected on three different days. The main reason for the increase in BOD and SS in the "wet" heading over the "dry" heading is that shrimp viscera are contained in the head cavity and, therefore, can be washed out of the head into the liquid effluent. The difference in BOD waste loads between "dry" and "wet" heading was statistically significant ($P < 0.1$). Also, the difference in SS waste loads between "dry" and "wet" heading was significant ($P < .005$).

The Federal Environmental Protection Agency's Development Document (4) outlines waste loading ratios for BOD and SS of 84 and 93 lbs/1000 lbs G.H., respectively, for the two breaded shrimp processing plants surveyed. Depending on size, breaded shrimp plants may process from 4,000 to 44,000 lbs G.H. shrimp per day. (5) Previous studies by this laboratory (3) on a large breaded shrimp plant in Georgia showed waste loading ratios on total BOD (expressed as lbs BOD/1000 lbs G.H.):

Before Hydroscreen	221
After Hydroscreen	121
After screening and dry clean-up	92

These figures illustrate that shrimp packing houses are totally different operations from shrimp processing plants. Packing houses handle an average of 200,000 - 300,000 lbs shrimp (heads-on) over the entire nine month season in Georgia. Corresponding daily average figures are 1010 - 1515 lbs/day for a packing house. Of this amount, approximately 60 - 70% of the catch is headed at sea to obtain higher prices. In contrast, shrimp processing plants use 10,000 - 20,000 lbs/day G.H. to manufacture breaded, cooked, and frozen products. The strongest waste load ratios obtained in the packing house samples collected are so low (BOD, 22 lbs/1000 lbs G.H. and SS, 7 lbs/1000 lbs G.H.) as to be insignificant

when compared to a processing plant. However, the amount of solid material (heads) to be disposed of can be much greater.

The percent reduction of loadings of BOD and SS obtained by screening are contained in Table 3. A reduction in the waste loads of BOD and SS after screening was not always obtained because of the fluctuation of the flow rate from the table. During this study, samplings of the liquid effluent after the screen were performed, then the liquid effluent before the screen was sampled. Flow rate was subject to small but eratic changes as piles of accumulated heads and associated ice were manually pushed into the flume.

The average percent reduction of waste loads from screening through 1/16, 1/4, and 1/2 inch openings screens using either the "dry" or "wet" heading technique are listed in Table 3. For the "dry" heading technique, the average reduction of BOD varied from an increase of 2.75% for the screen with 1/16 inch openings to a decrease of 6.75% for the screen with 1/2 inch openings. the average reduction of SS varied from an increase of 4.50% for the screen with 1/4 inch openings to a decrease of 15.25% for the screen with 1/16 inch openings. For the "wet" heading technique, the average reduction of BOD varied from 7.33% for the screen with 1/16 inch openings to 14.33% for the screen with 1/4 inch openings. The average reduction of SS varied from 7.67% for the screen with 1/16 inch openings to 15.67% for the screen with 1/2 inch openings. Note the large standard deviations of the average percent reductions. Only three of the determinations of average percent reduction listed in Table 3 were statistically significant ($P < 0.05$). The significant reductions were obtained in SS using the "dry" heading technique with a screen with 1/16 inch openings (16-18 mesh such as commonly used on screen doors) and in BOD and SS using the "wet" heading technique with a screen with 1/4 inch openings. The remainder of the reductions were not significant ($P < 0.05$). This represents a significant reduction in only 25% of the cases tested, and suggests that the screens are ineffective for removing solids other than heads, which typically measure 1.5 x 0.5 inch. Other than coarse screening is probably unnecessary, since loadings of BOD (9.1-17.4 lbs/1000 lbs G.H.) and SS (1.3-5.8 lbs/ 1000 lbs G.H.) are at such low ratios, whether using "dry" or "wet" heading techniques.

CONCLUSION

The findings of this study have shown that waste loads generated by shrimp packing houses are not as large as those from shrimp processors. The waste loading ratios from either heading technique ("dry" BOD and SS values of 9.09 ± 4.71 and 1.32 ± 0.18 lbs/

1000 lbs G.H., respectively, and "wet" BOD and SS values of 17.45 ± 4.38 and 5.81 ± 1.46 lbs/1000 lbs G.H., respectively) are ten-fold less than a typical processing plant producing breaded shrimp. Compare for example, the average plant in the federal study (4) which reported waste loading ratios for a breaded shrimp processor of 84 and 93 lbs/1000 lbs G.H. for BOD and SS, respectively. Another example is the previously cited large processing plant in Georgia, which generates a total raw waste load of 221 lbs BOD/1000 lbs G.H., but reduces this to 92 lbs/1000 lbs G.H. by screening and dry clean-up (3). This reduced load is still five to ten-fold higher than the average load from a packing house.

The lack of statistical significance in obtaining a reduction using any of the screens tested indicates that, for the sizes of screens tested (1/16, 1/4, and 1/2 inch openings), none were effective in removing any solids other than heads. Furthermore, the data on SS loads (1.3-5.8 lbs/1000 lbs G.H.) suggest that heads are probably the only solids in packing house effluents of any real significance. Thus, more stringent screening appears to be unnecessary.

Researchers from North Carolina State University have determined BOD and SS loads from a pilot plant study using the "dry" heading technique (7). They found the BOD and SS loading ratios of the unscreened effluent to be 4.79 and 1.22 lbs/1000 lbs G.H., respectively. After screening (3/8 inch openings), the loads of BOD and SS were 3.19 and 3.91 lbs/1000 lbs G.H. respectively (7). They found the waste load on a pilot scale to be even lower than the results we obtained on a full scale operation, which again confirms that the waste load from a shrimp packing house is low in comparison to processing operations.

The amount of solid material (heads) to dispose of can be much greater. Further studies were done to determine what happens to the heads, if put back in the water. The following report describes these results.

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ACKNOWLEDGEMENTS

TABLE 1

LOADING OF BOD AND SUSPENDED SOLIDS WITHOUT SCREENING FROM A SHRIMP PACKING HOUSE USING THE "DRY" HEADING PROCESS

No. of Sample[a]	Date	Heading Table Vol/day gal.	BOD	SS	Wash Vat Vol/day gal.	BOD lbs/day	SS	Total Discharged lbs/day BOD	SS	lbs BOD or SS per 1000 lbs G.H.[b] BOD	SS	Shrimp Headed 1000 lb G.H./day
3	9-19	8816	18.4	2.8	-	-	-	-	-	-	-	-
3	" "	8910	22.9	3.3	-	-	-	-	-	-	-	-
3	" "	9754	18.6	2.9	-	-	-	-	-	-	-	-
9	" Ave	9160	20.0	3.0	1150	1.5	0.2	21.5	3.2	7.68	1.14	2.798
1	9-20	1664	1.7	0.4	-	-	-	-	-	-	-	-
1	" "	1609	2.6	0.5	-	-	-	-	-	-	-	-
1	" "	1516	3.0	0.5	-	-	-	-	-	-	-	-
3	" Ave	1596	2.4	0.5	383	1.4	0.3	3.8	0.8	6.64	1.40	0.572
2	9-28	2885	5.5	1.2	-	-	-	-	-	-	-	-
2	" "	2885	9.1	1.2	-	-	-	-	-	-	-	-
2	" "	2737	7.9	1.5	-	-	-	-	-	-	-	-
6	" Ave	2836	7.5	1.3	383	2.3	0.3	7.8	1.6	5.96	1.22	1.309
2	9-29	2558	5.7	0.4	-	-	-	-	-	-	-	-
2	" "	2721	4.8	0.2	-	-	-	-	-	-	-	-
2	" "	2694	6.0	0.4	-	-	-	-	-	-	-	-
6	" Ave	2657	5.5	0.3	383	0.8	0.3	6.3	0.6	16.07	1.53	0.392
24	Ave All Days	4062	8.85	1.28	575	1.50	0.28	9.85	1.55	9.09	1.32	-
	Standard Deviation		7.10	1.12	-	0.62	0.05	7.94	1.18	4.71	0.18	-

a
Each Sample analyzed represents a composite of 5 grab samples taken within a 15 minute collection period during which 3 measurements of flow rate were also made and averaged. Compositing of samples from different 15 minute collection periods was done in proportion to the average flow rates of the respective periods.

b
Load ratio expressed as lbs. BOD or Suspended Solids per 1000 lb. Green Headless (G.H.) Shrimp headed.

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TABLE 2

LOADING OF BOD AND SUSPENDED SOLIDS WITHOUT SCREENING FROM
A SHRIMP PACKING HOUSE USING THE "WET" HEADING PROCESS

No. of Samples[a]	Date	Heading Table Vol/day gal	lbs/day BOD	SS	Wash Vat Vol/day gal	lbs/day BOD	SS	Total Discharged lbs/day BOD	SS	lbs BOD or SS per 1000 lbs G.H.[b] BOD	SS	Shrimp Headed 1000 lbs G.H./day
1	10-28	2460	2.8	1.0	—	—	—	—	—	—	—	—
1	"	2330	4.4	1.5	—	—	—	—	—	—	—	—
1	"	2071	8.9	3.9	—	—	—	—	—	—	—	—
3	" Ave	2287	5.4	2.1	383	1.6	0.3	7.0	2.4	18.42	6.32	0.380
1	11-05	3487	8.0	2.8	—	—	—	—	—	—	—	—
1	"	3371	9.3	3.1	—	—	—	—	—	—	—	—
1	"	3386	9.4	3.2	—	—	—	—	—	—	—	—
3	" Ave	3415	8.9	3.0	383	1.2	0.3	10.1	3.3	21.26	6.95	0.475
1	11-08	1649	3.1	1.1	—	—	—	—	—	—	—	—
1	"	1606	6.4	2.5	—	—	—	—	—	—	—	—
1	"	1463	8.0	2.8	—	—	—	—	—	—	—	—
3	" Ave	1573	5.8	2.1	383	1.8	0.4	7.6	2.5	12.67	4.17	.600
9	Ave. All Days	2425	6.7	2.43	383	1.53	0.33	8.23	2.73	17.45	5.81	—
	Standard Deviation	—	2.64	1.01	—	0.31	0.06	1.64	0.49	4.38	1.46	—

[a] Each Sample analyzed represents a composite of 5 grab samples taken within a 15 minute collection period during which 3 measurements of flow rate were also made and averaged. Compositing of samples from different 15 minute collection periods was done in proportion to the average flow rates of the respective periods.

[b] Load ratio expressed as lbs. BOD or Suspended Solids per 1000 lb. Green Headless (G.H.) Shrimp headed.
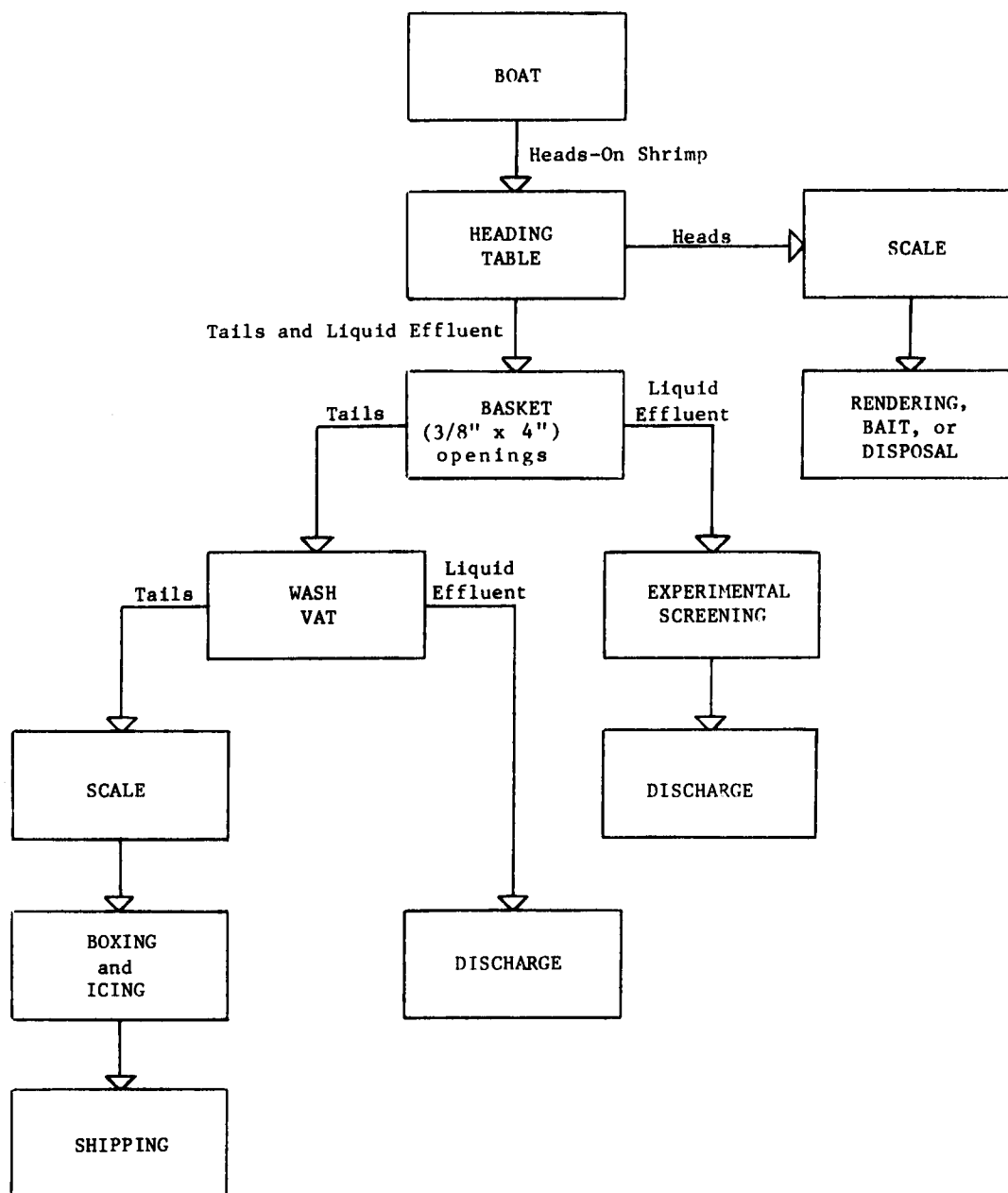
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Figure 1-A. Shrimp Packed By
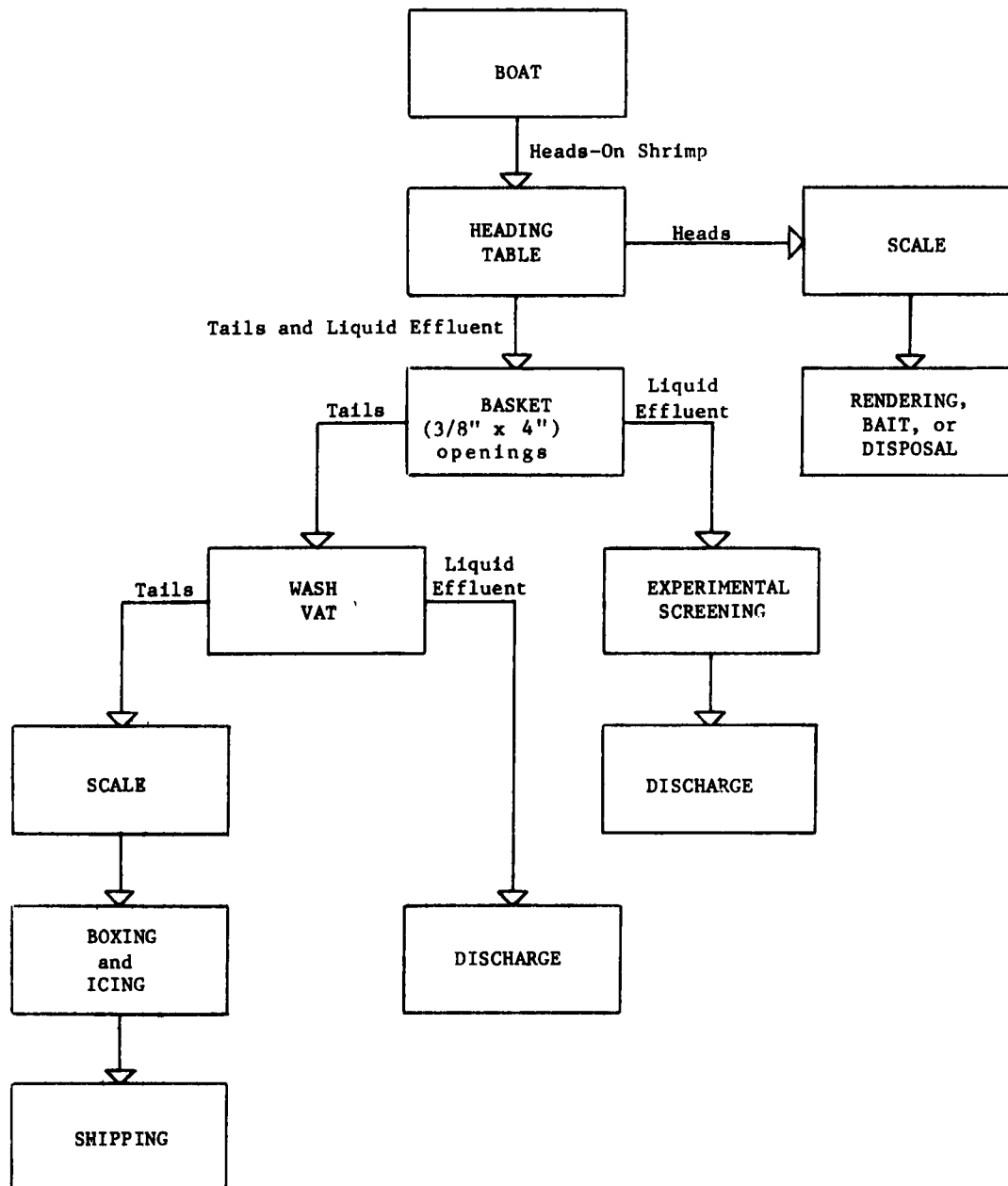The "Dry" Heading Technique

Figure 2-A. Shrimp Packed By
The "Wet" Heading Technique

ENUMERATION OF MICROBIAL FLORA IN SEAFOOD MARKETED IN FLORIDA
JANUARY 1, 1977 - DECEMBER 31, 1977

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INTRODUCTION

Seafood is a very perishable product which requires special
handling and special processing to maintain its quality. These
products contain the normal bacterial flora from their environ-
ments in addition to the contaminants acquired during harvesting
and handling. The initial flora of a product and the flora after
processing can indicate the shelf life of the product and
discrepancies in the processing procedures. Also, the flora can
indicate poor sanitation and possible mishandling of the product.
Therefore, it is necessary to know what the normal flora of a
seafood product is in order to judge the quality of seafood in
the market.

Aerobic plate count at 37° C, coliforms, E. coli, coagulase
positive staphylococci, Salmonella and organoleptic examination
are routinely used to judge the quality of fresh and frozen seafood.
Anaerobes are used for the quality of canned seafoods. There are
no universal microbiological standards for seafood, but there are
guidelines for judging the overall acceptability quality of seafood.
Perhaps quality is not an appropriate term to describe conditions
of the product, but it is the best term I could conjure. Florida
has no firm microbiological guidelines concerning seafood except
shellfish. Each sample is judged based on overall knowledge of
microbiological flora in the type of sample, organoleptic and
inspectional comments and findings. Some states and municipalities
have laws, regulations, codes or ordinances specifying microbiolog-
ical limits for seafoods.

General guidelines for quality will be briefly discussed with each
type of seafood. Table I is composed of states and municipalities
that have microbiological limits for seafood.

MATERIAL AND METHODS

Samples were collected by food inspectors during regular inspections. Perishable products were kept frozen or refrigerated until analyzed. Nonperishable products were stored at room temperature until they were analyzed. All analyses were performed in accordance with AOAC or BAM.

RESULTS AND DISCUSSION

A total of 565 samples of seafood products were analyzed. 421 samples were analyzed for aerobic plate count at 37° C, coliforms, E. coli, coagulase positive staphylococci and Salmonella. 144 samples were analyzed for anaerobes using thioglycollate 37° C and cooked meat 37° C. The samples were divided into eleven categories: fresh fish, canned fish, smoked fish, frozen breaded shrimp, frozen unbreaded shrimp, fresh crabmeat, pasteurized crabmeat, fresh oysters, frozen breaded scallops, frozen unbreaded scallops and miscellaneous seafood. Tables II through X display the levels of bacteria detected for the eleven categories of seafood. Table XI is a summary of the eleven categories of seafood. It displays the number of samples from each category, number of subquality samples, percent subquality samples, and the average percent of subquality for all the samples examined. Columns also indicate number of samples on which some type of regulatory action was required. Many of the microbiological levels cited to the processors could not be judged as indicating any health hazard and could probably best be judged as indicating quality lower than acceptable or possible mishandling during processing. Table XII, Summary of International Microbiological Criteria and Standards for Molluscan Shellfish shows the standards now in effect.

CONCLUSION

An average of 20.1% of seafood samples were found to be of subquality due to bacteria levels which suggest that better care is needed in handling seafood to insure the consumer a quality product.

REFERENCES

1. SPECK, M. L., 1976, Compendium of Methods for the Microbiological Examination of Foods.

TABLE I (CONTINUED)

STATES AND MUNICIPALITIES HAVING LAWS, REGULATIONS, CODES, OR

ORDINANCES SPECIFYING MICROBIOLOGICAL LIMITS FOR SEAFOODS (1975)

State or Municipality	Product	Microbiological limits	
Rhode Island	Seafood, fresh	Standard plate count	1,000,000/g
	Shellfish, fresh	Standard plate count	500,000/g
		Fecal coliform	2.3 for raw shellfish
	Smoked fish	Standard plate count	100,000/g
	Prepared foods, including seafood	Standard plate count	100,000/g
		Coliform organisms	100/g
	All prepared foods	Fecal streptococci	1000/g
		Staphylococci	100/g
		Staphylococcus aureus	none
		Salmonella and Shigella	none
		Beta hemolytic streptococci	none
		Vibrio parahaemolyticus	none
		Toxigenic mold	none

-NOTE: Action taken when arithmetic average of 10 samples are substandard or when 1 of 4 samples is substandard.

Virginia	Crabmeat	Standard plate count	100/g
		Fecal coliforms	50/100 g
		Coliform organisms	4900/g
	Oysters and clams	Standard plate count	500,000/g
		Fecal coliforms	230/100 g
Nebraska	Ready-to-eat foods	Standard plate count	100,000/g
		Coliform organisms	100/g
Chicago, IL	Shellfish	Standard plate count	500,000/g
		Fecal coliforms	230/100 g
St. Louis, MO	All foods	Standard plate count	$\leq$100,000/g
		Coliform organisms	$\leq$100/g
		Staphylococcus aureus	none
New York	All foods	Standard plate count	<1,000,000/g
		Escherichia coli	<3/g
		Staphylococcus aureus	<100/g
		Salmonella	none

Compendium of Methods for the Microbiological Examination of Foods, 1976

TABLE I

STATES AND MUNICIPALITIES HAVING LAWS, REGULATIONS, CODES, OR

ORDINANCES SPECIFYING MICROBIOLOGICAL LIMITS FOR SEAFOODS (1975)

State or Municipality	Product	Microbiological Limits	
Connecticut	Shellfish growing water	Coliform organism MPN	≤70/100 ml
	Shellfish	Standard plate count	≤500,000/g
		Escherichia coli	≤250/100g
Kentucky	Oysters	Coliform organism MPN	160,000/100 g
		Standard plate count	1,000,000/g
New York, N.Y.	Crabmeat	Staphylococcus aureus	<100/g
		Coliform organisms	<100/g
		Enterococci	<1000/g
		Standard Plate Count	<100,000/g
	Shellfish	Fecal coliforms	<230/100g
		Standard plate count	≤500,000/g
	Perishable or potentially hazardous foods*	Standard plate count	≤100,000/g
		Fecal coliforms	none
		Salmonella	none
Memphis-Shelby County, TN	Cooked foods	Standard plate count	100,000/g
	Seafood, uncooked	Standard plate count	1,000,000/g
Eau Claire City/ County, WI	Ready-to-eat foods	Standard plate count	100,000/g
		Coliform organisms	100/g
Georgia	Shellfish, etc.	Specified as "For use of State only."	
Indiana	Oysters	Escherichia coli	≤250/100g
		Standard plate count	≤500,000/g

*Includes ready-to-eat fish and crabmeat other than pasteurized or sterilized crabmeat.

Continued

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TABLE II

FRESH FISH

Number of samples examined	73
APC 37° C range/gm	0 - 4,200,000
Number of samples with SPC above 10^6	9
Coliforms range per gram	0 - 10,000
Number of samples with counts/g above 1000	2
E. coli range per gram	0 - 100
Number of samples with E. coli	1
Coagulase positive staphylococci	0 - 100
Number of samples with coagulase positive staphylococci above 100/g	6
Number of samples containing Salmonella	1

TABLE III

CANNED FISH

TYPE	NO. SAMPLES	APC 37° C RANGE	ANAEROBES +	-
Tuna	10	0	0	10
Sardines	16	0-600,000/gm	2	14
Mackeral	32	0	0	32
Anchovies	50	0	13	37
Herring	6	0-300/gm	2	4
Cuttle	3	0	0	3
Salmon	1	0	0	1
Mussels	2	0	0	2
Cod Fish	3	0	0	3
Salmon	4	0	0	4

TABLE IV

SMOKED FISH

Number of samples examined	19
APC 37° C range/gm	0 - 21,000,000
Number of samples with APC above 10^6	2
Coliforms MPN range	0 - 1000
Number of samples containing coliforms	3
Number of samples containing E. coli	0
Number of samples containing coagulase positive staphylococci	0
Number of samples containing Salmonella	0
Number of samples examined for water phase salt	19
Number of samples containing less than 3.5% water phase salt	4

TABLE V

FROZEN SHIRMP

	BREADED	UNBREADED
Number of samples examined	29	31
APC 37° C range/gm	3,000-80,000,000	12,000-90,000,000
Number of samples with APC above 10^6	12	7
Coliform range MPN	0-10,000	0-10,000
Number of samples with MPN above 1000/gm	1	4
E. coli range MPN	0-100	0-100
Number of samples with MPN above 10/gm	1	1
Coagulase positive staphylococci	0-1,100	0-210
Number of samples with MPN above 100/gm	2	1
Number of samples containing Salmonella	0	2 (E & G)

TABLE VI

FRESH CRAB MEAT

Number of samples examined	39
APC 37° C range/gm	3,000-10,000,000
Number of samples with APC above 10^6	6
Number of samples with APC above 10^5	11
Coliform range MPN	0-1100
Number of samples with MPN above 230	5
E. coli range MPN	0-3.6
Number of samples containing E. coli	4
Coagulase positive staphylococci range MPN	0-1100
Number of samples with MPN above 1000/gm	4
Number of samples that contained coagulase positive staphylococci	35
Number of samples containing Salmonella	0

TABLE VII

PASTEURIZED CRABMEAT

Number of samples examined	88
APC 37°C range/gm	0-310,000,000
Number of samples with APC above 3000/gm	17
Number of samples with APC above 10^6	8
Coliform range MPN	0-240
Number of samples with MPN above 230	1
E. coli range MPN	0-3.6
Number of samples containing E. coli	1
Coagulase positive staphylococci range MPN	0-1100
Number of samples containing coagulase positive staphylococci	6
Number of samples containing Salmonella	0
pH range	7.08-9.93

TABLE VIII

FRESH OYSTERS

Number of samples	41
APC 37°C range/gm	3,000-13,000,000
Number of samples with APC above 500,000/gm	6
Coliform range MPN	11,000
Number of samples with MPN above 230	16
E. coli range MPN	0-75
Number of samples with MPN above 3.6	6
Coagulase positive staphylococci range MPN	0-30
Number of samples with MPN above 0	4
Salmonella	0
pH range	5.84-6.55
Number samples with pH below 6.0	7

TABLE IX

FROZEN SCALLOPS

	BREADED	UNBREADED
Number of samples examined	6	19
APC 37°C range/gm	3,000-56,000	3,000-25,000,000
Number of samples with APC above 10^6	0	7
Coliforms range MPN	0-100	0-1100
Number of samples with MPN above 230	0	3
E. coli	0	0
Coagulase positive staphylococci	0-93	0-100
Number of samples containing coagulase positive staphylococci	3	3
Salmonella	0	0

TABLE X

MISCELLANEOUS SEAFOOD

Number of samples examined	93
APC 37°C range/gm	0-48,000,000
Number of samples with APC above 10^6	17
Coliform range MPN	0-10,000
Number of samples with MPN above 1000	5
E. coli range MPN	0-10,000
Number of samples with MPN above 100	3
Coagulase positive staphylococci range MPN	0-460
Number of samples containing coagulase positive staphylococci	16
Number of samples containing Salmonella	1

TABLE XI

<u>S U M M A R Y</u>

PRODUCT	NUMBER OF SAMPLES EXAMINED	NUMBER OF SUB-QUALITY SAMPLES	% SUB-QUALITY SAMPLES
Fresh Fish	73	11	15.1
Canned Fish	127	17	13.4
Smoked Fish	19	2	10.5
Frozen Shrimp (unbreaded)	31	9	29.0
Frozen Shrimp (breaded)	29	12	41.4
Fresh Crabmeat	39	13	33.3
Pasteurized Crabmeat	88	9	10.2
Fresh Oysters	41	16	33.3
Frozen Scallops (unbreaded)	19	7	36.8
Frozen Scallops (breaded)	6	0	0
Miscellaneous Seafood	93	18	19.4
TOTAL	565	114	20.11

TABLE XII

SUMMARY OF INTERNATIONAL MICROBIOLOGICAL CRITERIA AND

STANDARDS FOR MOLLUSCAN SHELLFISH

| COUNTRY	MICROBIOLOGICAL CRITERIA	MICROBIOLOGICAL STANDARD
Federal Republic of Germany	1. Total number of bacteria 2. Coliform Score 3. Presence of Pathogenic bacteria	

Canada
Japan
Korea
New South Wales
	1. Fecal Coliform MPN
	
	2. Standard Plate Count/gm
		1. Satisfactory-230/100 gms.
		  Conditional->230/100 gms.
		2. Satisfactory-500,000/gm.
		  Conditional->500,000/gm.

United Kingdom
Holland
Belgium
	1. Fecal E. coli
	  Roll tube count
		1. Acceptable--90% up to 200
		        per 100 ml
		        10%-200 to 500
		        per 100 ml
		  Unacceptable->500 per
		        100 ml

France	1. E. coli MPN	1. Oysters and molluscans
		  ordinarily eaten raw -
		  <1 per ml
		  Mussels and molluscs
		  ordinarily eaten cooked-
		  not >2 per ml.

Denmark	1. E. coli Type I plate count
	
	2. Plate Count per gram
	
	3. Salmonella
		1. Each of 10 shellfish
		  should be negative.
		2. Each of 10 shellfish
		  should not exceed 100,000/g
		3. Each of 10 shellfish
		  should be negative.

Italy	1. E. coli MPN	1. Approved Area Shellfish:
		  -160 per 100 ml of sample
		  shall not be exceeded in
		  90% of samples during
		  one year.
		  -500 per 100 ml of sample
		  shall not be exceeded in
		  10% of samples during
		  one year.
		  Market Shellfish:
		  -600 per 100 grams of
		  sample shall not be exceeded

Sweden	1. Same as for countries importing Swedish shellfish.	1. Same as for countries importing Swedish shellfish

Compendium of Methods for the Microbiological Examination of Foods, 1976

FACTORS AFFECTING PROTEOLYTIC BREAKDOWN OF TEXTURE IN MINCED FISH GELS
A PROJECT STATUS REPORT

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With the introduction of mechanical deboners to the fishing in-
dustry, an economical means of recovering large quantities of edible
fish flesh was made possible. As prices of other muscle foods has
risen, interest in fabricating new foods from this relatively cheap
resource has also intensified. Recent attempts to produce processed
fish products of thé gel or emulsion type have shown a heavy reliance
on use of additive binders such as textured vegetable proteins to
insure firm texture in the processed product. This research was insti-
gated as a basic study into the factors which affect the texture of
gels from minced fish in hopes that a basic understanding of these
factors might enable greater control of quality in the production
of minced fish products without as much reliance on additive binders.

The muscle tissue of fish, as well as other animals, can be
fractionated based on solubility of the component proteins according
to the following scheme:

FISH MUSCLE TISSUE

water → sarcoplasmic proteins

2-3% NaCl → myofibrillar proteins
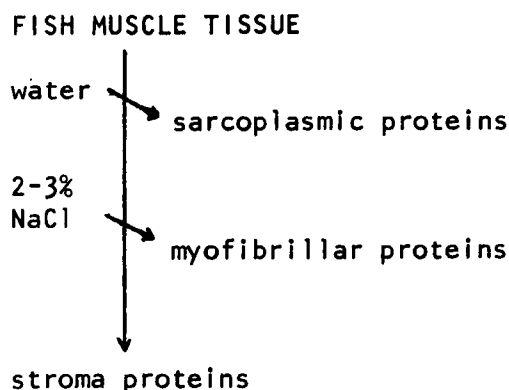
stroma proteins

The water-soluble, or sarcoplasmic, protein fraction contains the
enzyme-rich sarcoplasm of the muscle cell. The insoluble stroma pro-
tein fraction is composed largely of connective tissue. Neither
of these fractions is as critical to textural binding of gel or emul-
sion type products as the salt soluble or myofibrillar protein fraction,
however, which is composed of the proteins from the muscle myofibrils
and constitutes the bulk of the protein contained in fish muscle.

The basic fish gel used for analysis in this research was prepared from frozen, deboned fish which was tempered and broken into chunks prior to chopping in a silent cutter with salt addition to about 1°C in order to extract the myofibrillar proteins. This action resulted in a finely comminuted meat mass which could be stuffed into pans or casings and heat processed to yield a completely cooked gel product.

The texture of the cooked gel was measured both instrumentally, by means of a Kramer shear press and by dynamic compression, and by a texture profile panel, patterned after a similar panel developed at General Foods, Inc.

In the initial work of this study, deboned flesh was obtained from from species originating in the Gulf of Mexico and the textural properties of the gels prepared from the different tissues compared. As shown by the shear press data on these gels (Figure 1), gels pre-pared from sea trout were superior in textural properties to those prepared from croaker, mullet and ribbon (cutlassfish). In an attempt to explain these differences, the protein composition of the four species was compared by gel electrophoresis and by various analyses of the cooked gels. It was subsequently found that little or no differences could be found in the protein composition of the four raw tissues; thus, textural differences could not be explained in terms of differing quant-ities of salt-soluble proteins. However, analyses of the cooked gels revealed a lower water holding capacity and a higher percentage of ex-tractable protein (3% salt) in the poorer textured gels. The latter observation suggested that proteolysis was occurring during thermal processing of the gels prepared from croaker, mullet and ribbon. Follow-up studies revealed that the proteolytic factor responsible, possibly an enzyme, could be inactivated by rapid heating to 85°C and the texture improved, while gels cooked for one hour at 55°C or 70°C showed poor texture and subsequent electrophoresis revealed significant breakdown of several key myofibrillar proteins. Trout did not appear to contain the proteolytic factor, however, producing gels of equally good texture at all processing temperatures.

Using an assay procedure with casein as subtrate, proteolytic activity of a crude extract from croaker was measured at various pH values and temperatures (Figures 2 and 3). Results revealed a temperature optimum near 60°C and a pH maximum over the range 8.0-8.5. Fractionation of the muscle according to Figure 1 and subsequent assay revealed that the proteolytic factor resides mainly in the water-soluble fraction.

In a cooperative study with NMFS in Pascagoula, Miss., croaker from the Gulf were compared with croaker caught off the North Carolina coast in a similar manner to the earlier work. Initial results showed a superiority in textural attributes in the gels prepared from North Carolina croaker (Table 1), especially when the gels were held at 70°C for one hour prior to heating to 85°C. These differences were charted over a 12-month period with sampling at each season. Results revealed that the North Carolina croaker maintained this superiority overall

(Figure 4). The correlation coefficients (R^2 values) between various textural measurements and measurements of the chemical properties of the gels are shown in Table 2. Again, a high correlation was found between textural properties and water holding capacity and protein solubility of the cooked gel. The percent myosin (a major myofibrillar protein) retention, as measured from electrophoretic measurements, was also found to correlate highly with textural properties. However, alkaline protease activity, as measured by the casein substrate method mentioned previously at pH 7.0 and 60°C, correlated poorly with textural properties of gels. This indicates that either the wrong proteolytic factor was being assayed or that the assay conditions used do not reflect actual conditions within the gel.

These differences between North Carolina and Gulf of Mexico croaker were initially postulated to be due to differences in time of holding at the two locations. An experiment was conducted in which fish were obtained which had been iced for two days with half the lot subsequently headed, eviscerated and scaled. These two lots were then iced for eight additional days and then initial and final samples, held frozen after sampling, were thawed, the remaining fish cleaned, and deboned mechanically. Comparison of the four resulting samples (Table 3) revealed greater differences in protein extract-ability in the cooked gel between eviscerated and non-eviscerated fish than between 2-day old fish and 10-day old fish. These relationships were borne out in texture measurements also. Thus, it would appear that evisceration may be more critical in control of the level of the proteolytic factor in fish tissue than is age of the iced catch.

Because the proteolytic factor is present in the water soluble fraction of fish muscle it can be surmized that washing of deboned flesh could significantly reduce the level of this factor. Preliminary studies in this laboratory have shown this to be true, in agreement with the industrial ex-perience of Japan where extensive washing of fish flesh has long been a necessary step in the production of high quality "Kamaboko," a fish sausage product. As washing of deboned flesh has been recommended by several researchers to remove objectionable pigmentation and improve storage life, improvement of texture by removal of this degradative proteolytic factor may be an added benefit. An extensive study is under-way in this laboratory at the present time to evaluate fully the benefits to be derived from washing of deboned fish flesh.

In conclusion, it has been shown that a proteolytic factor is pre-sent in the flesh of certain fish species which degrades the texture of fish gels at normal processing temperatures. Complete characterization of this factor is now underway in this laboratory. Presently, the effects of this factor may be controlled by rapid heating to elevated temperatures (85°C) for products of small size dimensions. Eventually washing of fish flesh and/or better control of handling practices may offer the best means for control of texture in fish gels. Gel products offer a vehicle for large scale utilization of underutilized fish species and scrap; several products have been developed in this laboratory having excellent

textural properties and taste appeal including frankfurters, luncheon meats, salads and dips, jerky and salad toppings, all based on the basic fish gel described above. These products were prepared without use of additive binders, save the addition of 1-3% nonfat dry milk solids. Conceived as product innovations, not product imitations, these delicate smoked-seafood flavored products are natural additions to existing product lines, offering superior nutritional characteristics as well as a competitive price. Two publications will be available by mid-1978 from North Carolina Sea Grant to fish and meat processors to promote the production and utilization of minced fish: (1) "An Annotated Bibliography on Mechanically Separated Finfish and Crustacea Meats" and (2) a booklet entitled "Minced Fish: Its Production and Use," which outlines raw material sources, available equipment, quality control, end uses and cost factors for every phase of minced fish manufacturing. A condensed slide-tape presentation of the latter publication is also planned and should be available by late fall of 1978. Further research into factors affecting functionality of fish proteins in new products by this and other laboratories should provide the sound basis for investments into production and use of minced fish in the coming years.

Table 1. Comparison of physical and chemical parameters of texture in gels prepared from North Carolina and Gulf of Mexico croaker.[a]

	% Myosin	MSF[b]	Panel Elasticity	Panel Gumminess	WHC[c]
N. C. Croaker					
70-85°C	70.09	5.53	9	1	2.54
85°C	82.1	5.82	10	1	3.0
Gulf Croaker					
70-85°C	23.42	1.71	3.3	9	12.58
85°C	59.57	3.12	7	2.7	3.02

[a]Mean of 3 determinations.

[b]Maximum Shear Force.

[c]Water holding capacity, defined as water released during centrifugation.

Table 2. Correlation coefficients (R^2) relating chemical and physical attributes of gels prepared from minced croaker during a one year study.

| | | GEL CHARACTERISTICS | | | | | |
| | | Desirable | | | | Undesirable | |
	Max. Shear Force	Spring- iness	Firm- ness	Cohesive- ness	Adhesive- ness	Coarse- ness	Graini- ness
WHC	.77	.82	.80	.80	-.79	-.82	-.74
Prot. Sol (Cooked Gel)	-.79	-.77	-.75	-.75	.88	.79	.73
% Myosin Ret.	.89	.93	.92	.91	-.86	-.86	-.89
Alk. Protease Activity	-.14	-.08	-.17	-.12	.01	-.06	.12

Table 3. Protein solubility (5 mM Tris) of the cooked gels prepared from eviscerated and non-eviscerated fish frozen at time of evisceration and after eight days ice storage.

| | Protein Solubility (Cooked Gel) | |
| | Days in Ice | |
	0 days	8 days
Eviscerated	1.01	1.91
Non-eviscerated	6.03	7.46
	(slow cook)	
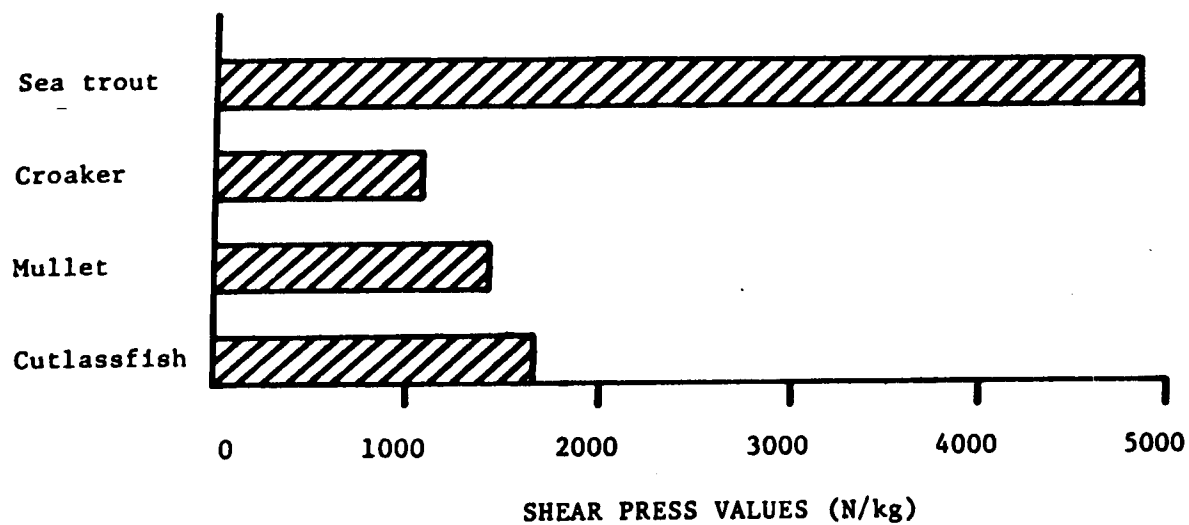
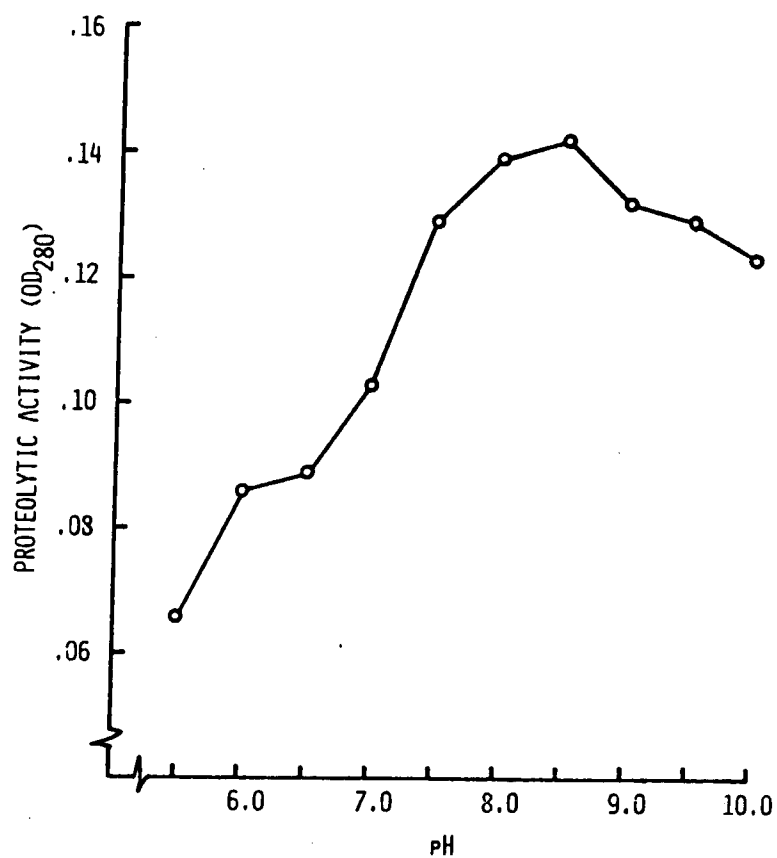Figure 1. Shear press values of gels prepared from four Gulf of Mexico
fish species.

Figure 2. pH activity curve for proteolysis of casein by croaker extract at 60°C.
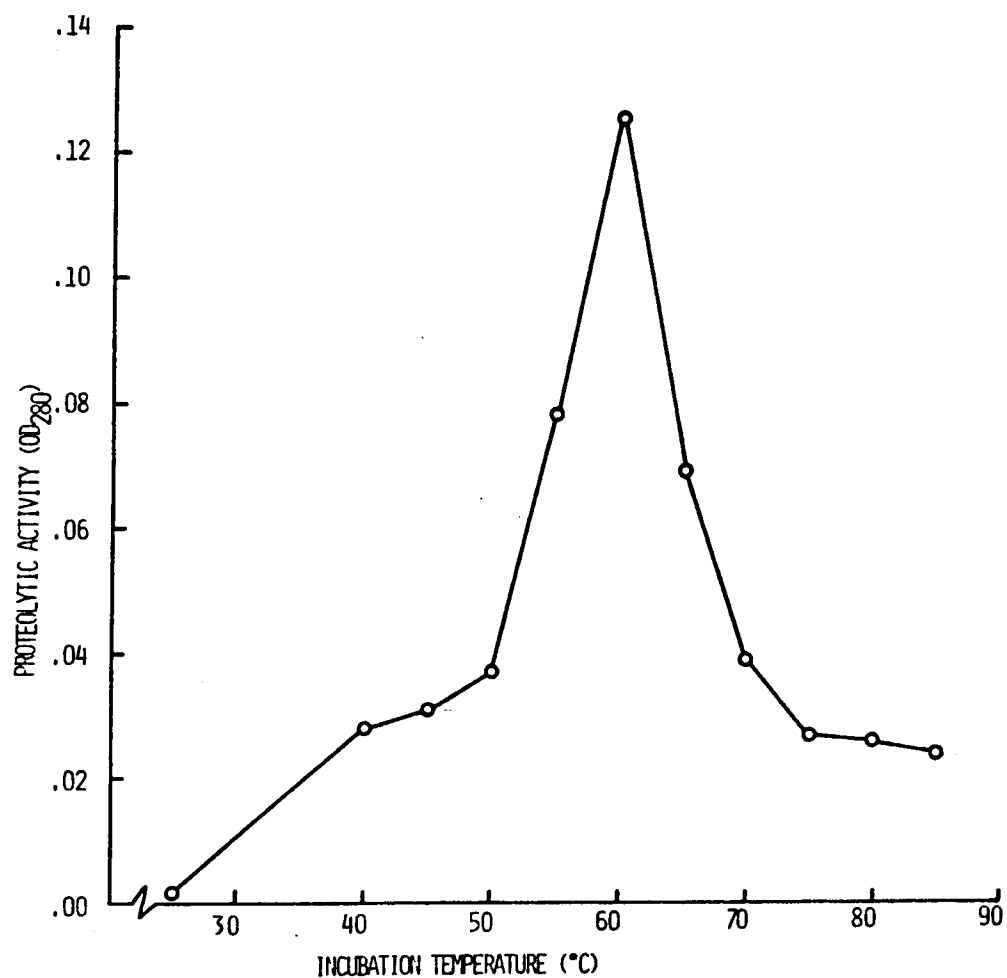
Figure 3. Temperature activity curve for proteolysis of casein by croaker extract at pH 7.5.
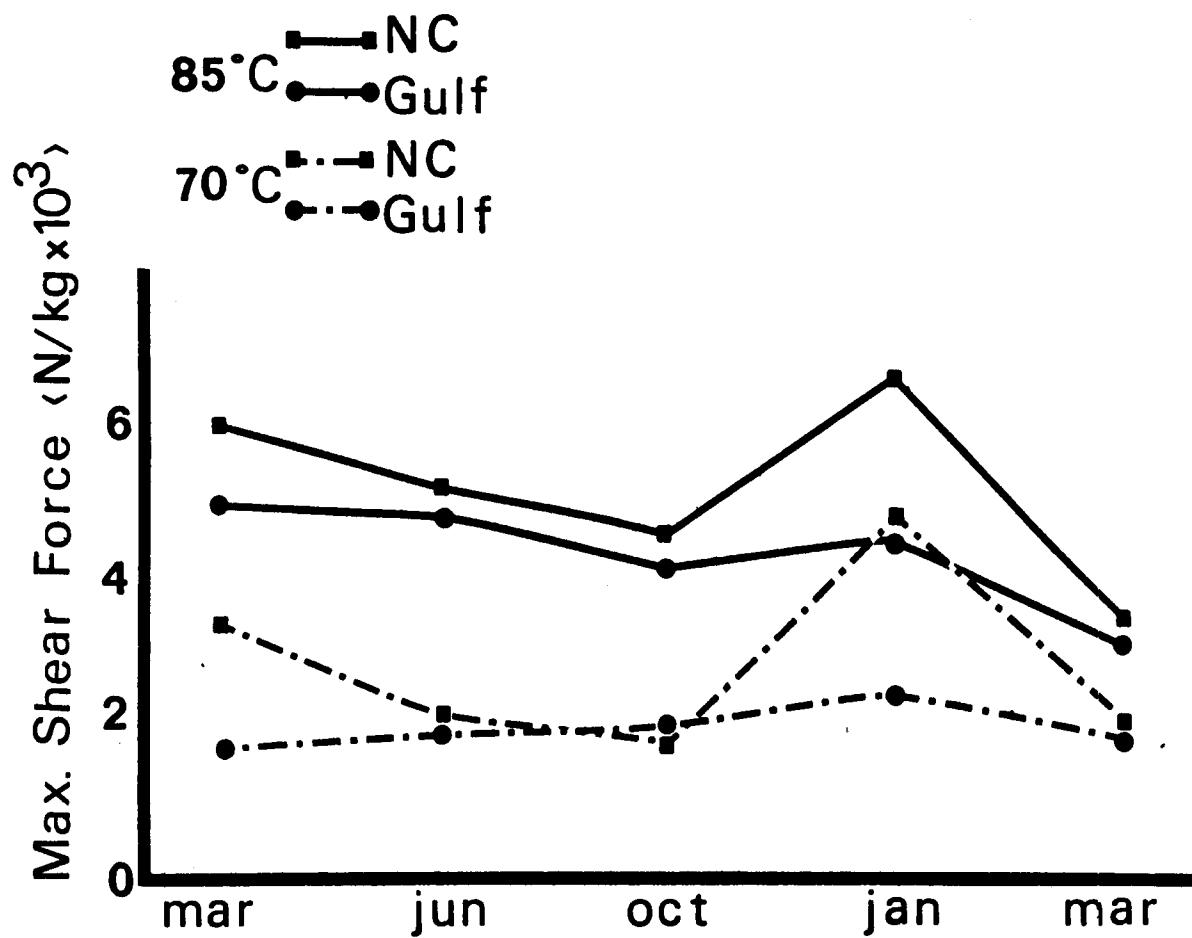
Figure 4. Comparison of texture in gels prepared from North Carolina and Gulf of Mexico croaker over a one year period.

Flavor Losses in Mechanically-Picked Crab Meats

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Mechanization of the seafood industry has long been an expensive and not too realistic goal. The Blue Crab industry, like other seafood producers, have been and are for the most part interested in updating and mechanizing their operations. Until recently mechanization had been a near impossibility. A mechanical crab meat extractor has been developed, which if adopted and utilized, will revolutionize the crab industry.

Preliminary purchasers of this machine have found difficulty in selling the meat for several reasons: (1) The psychological feelings of the public to the idea that hand-picked crab meat is of superb quality, (2) the loss of lump meat during the machine picking of the meat, and (3) the meat which is picked by machine is almost completely devoid of flavor.

The problems can be overcome by either modifying the processing methods or by reeducating the public in their ideas about crab processing. The loss of lump meat can be minimized by either selectively removing the large lump after it has been machine-picked. And finally, even if the machine-picked lump is not removed from the flake meat, the savings in labor alone can compensate for the losses in lump meat sales.

This work was undertaken to assess the differences between hand- and mechanically-extracted meats. First objective was to determine if any flavor differences existed and if so what was the extent of the differences. After assessing the differences, attempts were made to modify the flavor of mechanically-picked meats so that their flavor had relatively the same intensity as that of hand-picked meats. Finally, the chemical compositions of the two meats were looked at to determine what the actual causes of the flavor loss in mechanically-picked meats were.

All meat used in this study was fresh and obtained from a Louisiana crab producer who was processing with both hand pickers and the mechanical extractor.

Preliminary organoleptic analysis of the meats was carried out by the procedures described by Amerine et al. (1). An eight member panel scored the flavor, texture and odor of the meats.

The scores of the organoleptic analysis are shown in Table 1. For all attributes the scores of the mechanically-picked meats were judged significantly lower than the hand-picked meats. Flavor scores displayed the broadest variation, having been described as bland. These results are similar to those of Tinker et al. (3).

In attempting to improve the flavor of hand-picked crab meat, dip solutions were employed using the procedures of Tinker et al. (3). These did not improve the flavor to an acceptable level. Modification of the dip solutions likewise did not improve the flavor. By using the dip solutions as final cook solutions, improvement of the flavor was possible. Table 2 shows the results of organoleptic tests using the final cook solutions. Meat was given a final cook by placing in the boiling solution for 5 minutes.

All cook solutions have significantly higher scores than the un-treated meats. The untreated meat was given a final cook in boiling water with no additives. Solution A, which consisted of 0.15% salt, 0.25% MSG and 0.25% Ribotide gave the most improved flavor. The higher flavor scores for the crab meat using the cook solutions as yet cannot be explained.

Bacteriological analysis of the crab meats showed the mechanically-picked meats to have both lower total counts and coliform counts than the hand-picked meats. These results are comparable to those of Tinker et al. (3), and probably arise from the fact that the mechanically-picked meats are handled much less than the hand-picked meats, and also that the final cook acts as a pasteurization step.

Analysis of selected minerals in the crab meat by the methods of A.O.A.C. (2) showed significant differences in the levels of phosphorus, copper, potassium and sodium (Table 3). This is probably due to the solubility of these minerals in water, however, this is still being studied.

This preliminary work has shown that there is indeed a flavor loss in machine-picking of Blue crab meat, and that this flavor loss is directly related to the amount of water used in the processing. The work has further shown that the use of final cook solutions, containing salt, Ribotide and MSG, will greatly improve the flavor of the machine-picked crab meat.

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2. Association of Official Analytical Chemists. 1970. Official Methods of Analysis. 11th Ed. A.O.A.C., Washington, D.C.

3. Tinker, B. L., V. G. Ampola, K. A. Wilhelm and R. J. Learson. 1975. 1975 Progress Report on Blue Crab Research. U.S. Department of Commerce. N.M.F.S., Gloucester, Mass.

TABLE 1. PRELIMINARY ORGANOLEPTIC SCORES FOR CRAB MEAT

	ODOR	FLAVOR	TEXTURE
Hand-Picked	6.85	7.31	6.00
Machine-Picked	4.54	3.54	4.38

TABLE 2. FLAVOR SCORES FOR CRAB MEAT COOK IN
VARIOUS FINAL COOK SOLUTIONS

COOK SOLUTION	FLAVOR SCORE
Hand-Picked	3.73
1.5% NaCl/.25% MSG/.25% Ribotide[1]	5.42
1.5% NaCl/.25% MSG/.125% Ribotide	4.82
1.5% NaCl/.125% MSG/.25% Ribotide	5.25
1.5% NaCl/.125% MSG/.125% Ribotide	5.19
Untreated	2.76

[1]Ribotide is a mixture of ribonucleotides produced by Takadeo.

TABLE 3. MINERAL COMPOSITION OF BLUE CRAB MEATS

	HAND-PICKED	MACHINE-PICKED
Ca	.13%	.17%
P	1.28%	.57%
Cu	34.93 ppm	10.63 ppm
K	1.22%	.22%
Fe	17.27 ppm	18.70 ppm
Na	.53%	.10%

STORAGE CHARACTERISTICS OF SEVERAL UNDERUTILIZED
FISH FROM THE GULF OF MEXICO[1]/

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INTRODUCTION

The underutilized fishery resources of the United States have
taken on added significance since passage of the Fishery Conservation
and Management Act of 1976. The Act prohibits foreign fishing within
200 miles of the U.S. coastline except by international agreement.
The underutilized resource became more important economically to
the U.S. fishing industry due to restricted foreign fishing, which
supplied these countries with large volumes of product for export
and domestic use. The curtailment of fishing by foreign fleets
places added responsibility on the U.S. Government, industry, fishery
trade organizations, and others, to fully develop the fishery resources
to supply domestic and foreign demand. It is expected that the United
States will be called on to supply a greater portion of the world's
fishery needs. By the same token, U.S. fishing will be somewhat
limited by similar restraints placed on it by foreign countries with
similar laws. For example, the Florida lobster fishery has been
almost destroyed by restraints placed on it by the Bahamian Government.
Further, based on current Mexican policy, unless new agreements are
negotiated, by the end of 1979, shrimp fishing in Mexican waters
will be prohibited. Tuna, grouper, and snapper fishing in waters
off the western coasts of Mexico and South and Central America has
been drastically reduced due to fishing rights employed by those
countries. As U.S. landings of traditionally highly sought-after
species are reduced, greater pressure will be brought to bear on
other species and particularly on underutilized resources to meet
the demand. The U.S. fishing industry must be ready to supply this
demand for fishery products or it will be threatened with allowing
foreign firms an "in" in harvesting and utilizing the resource which
the 200-mile law now protects. In short, the industry must use it
or lose it.

The proper and orderly development of the underutilized resource
is very important to the economy of the United States, particularly
in the Southeast where groundfish and pelagic finfish resources are
abundant. While the region already supports important fisheries,
considerable need exists to diversify, to develop new approaches,
and to establish new industries.There are about 175 species of
groundfish and 11 major species of pelagics remaining to be fully

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[1]/Contribution No. 78-40P

used. Although the groundfish resource is utilized more extensively, the pelagics, other than menhaden, remain essentially untouched. If these stocks are effectively utilized, annual sales will increase substantially and create many new jobs.

The most important phase in the development of food markets for underutilized stocks includes proper handling, processing, and storage of the product. The production of high quality products is essential to the success of the utilization effort, particularly if the products are for export to countries imposing rigid quality standards.

Product quality and storage characteristics for many of the southeastern underutilized species have not been adequately assessed and documented in the literature. The shelf life of various product forms has not been determined but is important to management in deciding whether to enter the market (domestic and foreign) with new products. To establish this information through scientific methods is the first step in providing the seafood industry with the knowledge necessary to make sound decisions.

In an effort to meet our obligation to provide basic information to the industry for developing the resource, we of the SEFC, Charleston Laboratory, are conducting a series of experiments designed to assess the fresh and frozen shelf life and storage characteristics of several underutilized species. Species selected for study were those of greatest potential to the industry and those that would be of immediate benefit in an expanded utilization effort. Refrigerated product storage studies conducted and completed to date, and which I now report on, include Spanish mackerel *(Scomberomorous maculatus)*, king mackerel *(Scomberomorous cavalla)*, croaker *(Micropogon undulatus)*, and white trout *(Cynoscion arenarius)*.

MATERIALS AND METHODS

Fish Samples

The fish studied were obtained as fresh as possible and for the most part from a local seafood dealer. In all instances, the fish had been harvested no more than 3 days when processed in our laboratory. Processing consisted of hand-scaling, heading, gutting, filleting, skinning, and steaking. Each species was processed into product forms that would be most suitable to the consumer and most adaptable to the type of fish under study. Croaker, white trout, and Spanish mackerel were prepared and stored as whole fish, headed and gutted (H&G) fish, and fillets; king mackerel was prepared as whole fish and as steaks. Headed and gutted fish, fillets, and steaks were placed on polystyrene food trays and overwrapped with polyvinyl chloride packaging material. Whole fish were packed in crushed ice and all product forms held in a cooler maintained at 4°C. Representative samples of each product form were removed from storage at regular intervals and evaluated by chemical, microbial, and organoleptic methods.

Product Evaluation
Microbial analyses consisted of determining the total aerobic plate count as outlined in FDA's Bacteriological Analytical Manual for Foods (1976) (6).

Organoleptic quality was assessed by six experienced panelists evaluating the raw products for odor, texture, and appearance. Whole and H&G fish were assessed for odor as they were stored. Fillets from these fish were assessed for texture and appearance. A scale of 1 to 9 was used to rate the products: 9 representing excellent quality and 1 representing inedible quality.

Chemical analyses included analysis for oxidative rancidity using the TBA method as described by Vynche (7); Total Volatile Nitrogen (TVN) and Trimethylamine-Nitrogen (TMA-N) as developed by Cobb, *et al.* (5); pH and moisture content by the method of the AOAC (1).

Microbial and chemical analyses were conducted in triplicate and the results reported as an average.

RESULTS AND DISCUSSION

The results showed very good correlation between the test used. For example, when a significant change occurred in the values of the chemical tests, the organoleptic and microbial results generally followed the same pattern.

The Conway microdiffusion method was used to determine TVN and TMA-N. We are very pleased with this technique, because it is easy to conduct, and a large number of samples can be analyzed in a minimum amount of time. Additionally, results of the TVN analysis correlated extremely well with organoleptic assessments. For example, immediately after th panel reported the presence of slight spoilage odors in fillets, the TVN increased significantly and continued to increase until the product was judged spoiled. The TVN content of the various forms of croaker, white trout, Spanish mackerel, and king mackerel during refrigerated storage is shown in Figures 1, 2, 3, and 4. It can be observed that the TVN content increased significantly for: (1) H&G croaker and croaker fillets after 5 days of storage, (2) H&G white trout, skin-on and skinless trout fillets after 6 days of storage, (3) skin-on and skinless fillets of Spanish mackerel after 5 days of storage, and (4) king mackerel steaks after 9 days of storage. Considering all species evaluated, the TVN content did not exceed 16 mg N/100 g of sample until after the panel reported slight spoilage odors. Generally speaking, the results show that when the sensory panel considered the product spoiled, the TVN values ranged from about 23 to 28 mg N/100 g of sample.

The results show that TVN values for fillets and steaks increased more readily than H&G fish, and as a rule values were higher for H&G fish than whole fish. Values for skin-on fillets were slightly higher than those for skinless fillets for species where both products were evaluated. Because of the higher TVN
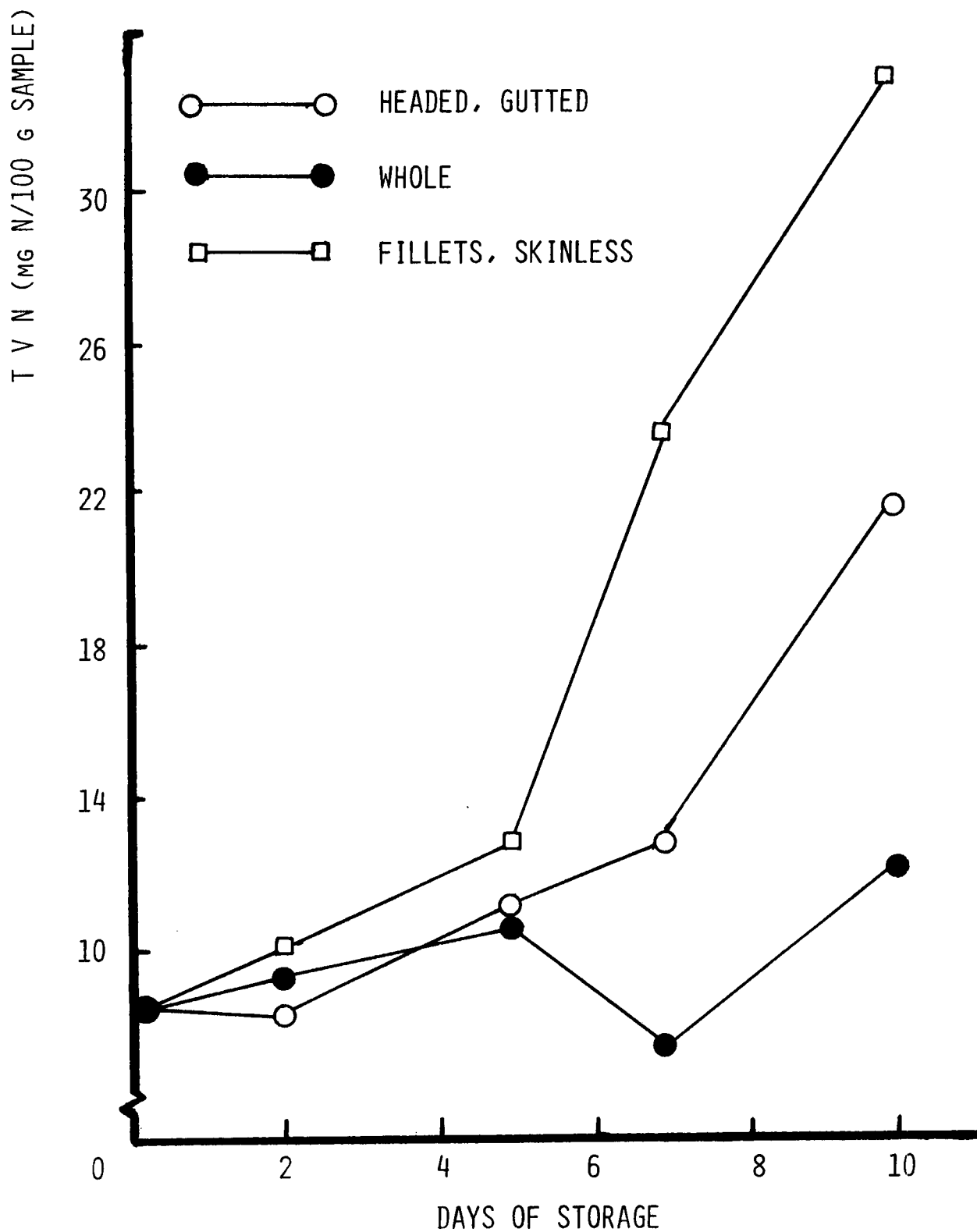
256

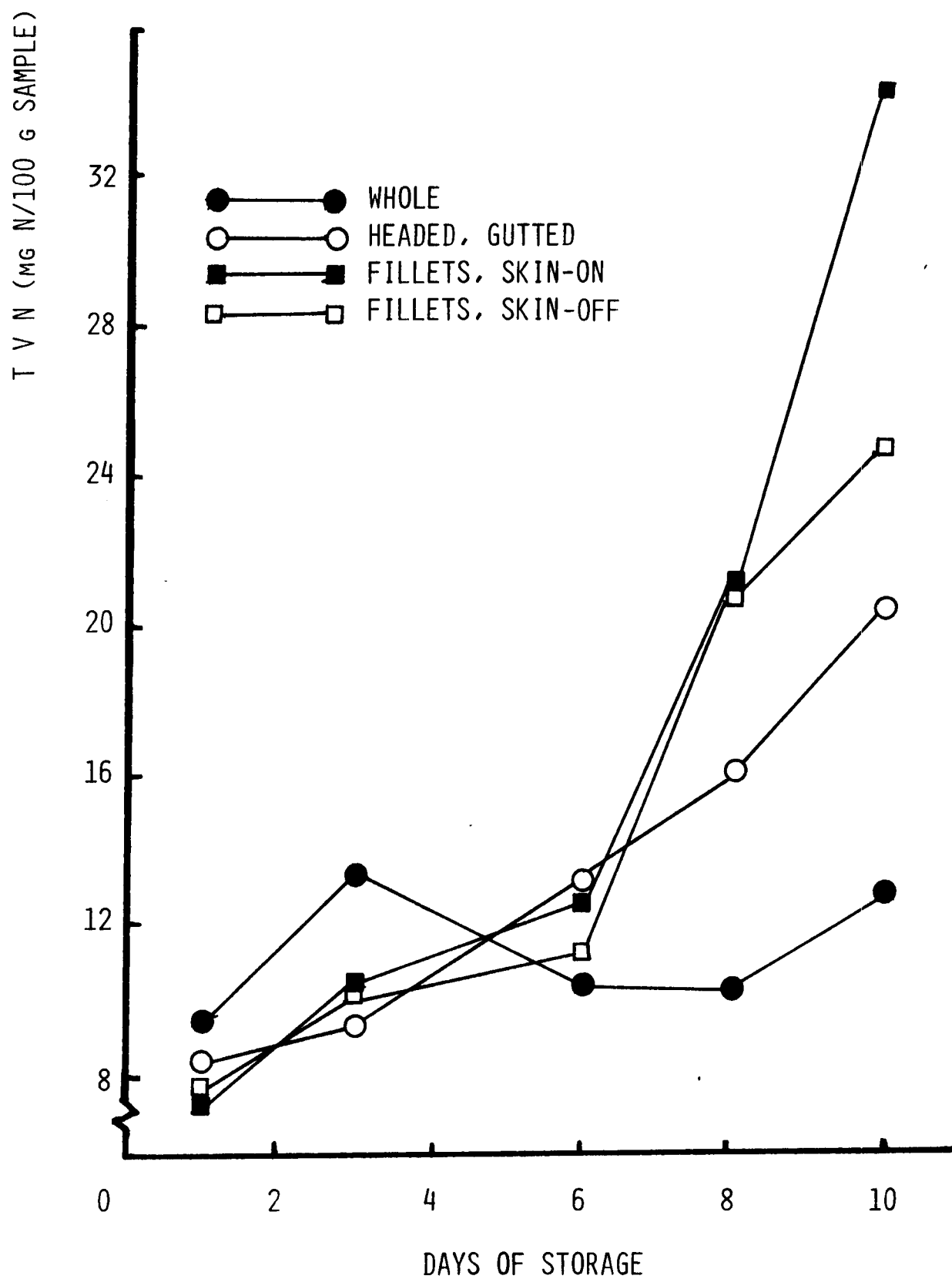FIGURE 1: TOTAL VOLATILE NITROGEN CONTENT OF CROAKER DURING STORAGE AT 4°C.

FIGURE 2: TOTAL VOLATILE NITROGEN CONTENT OF WHITE TROUT DURING STORAGE AT 4°C.
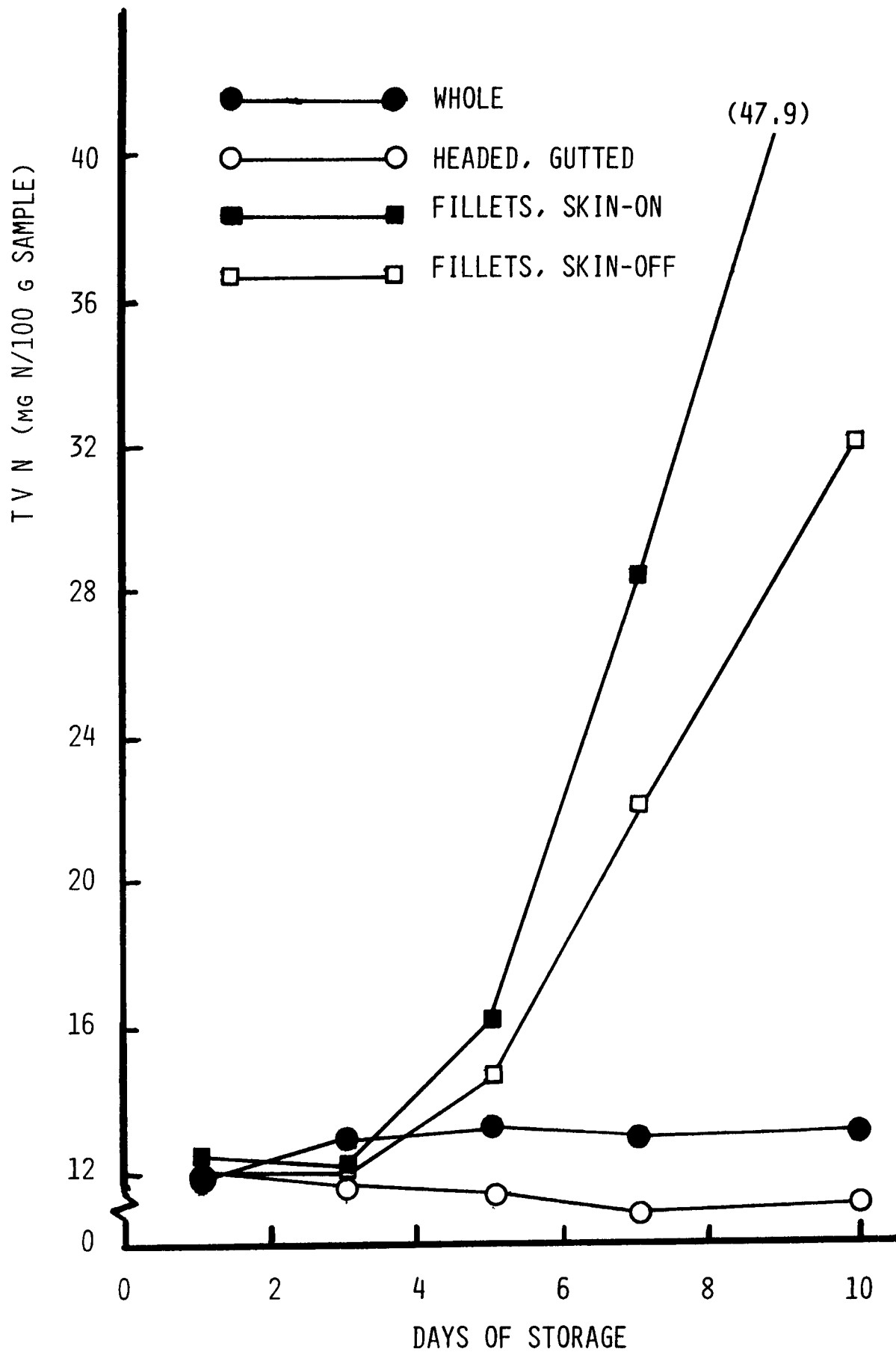
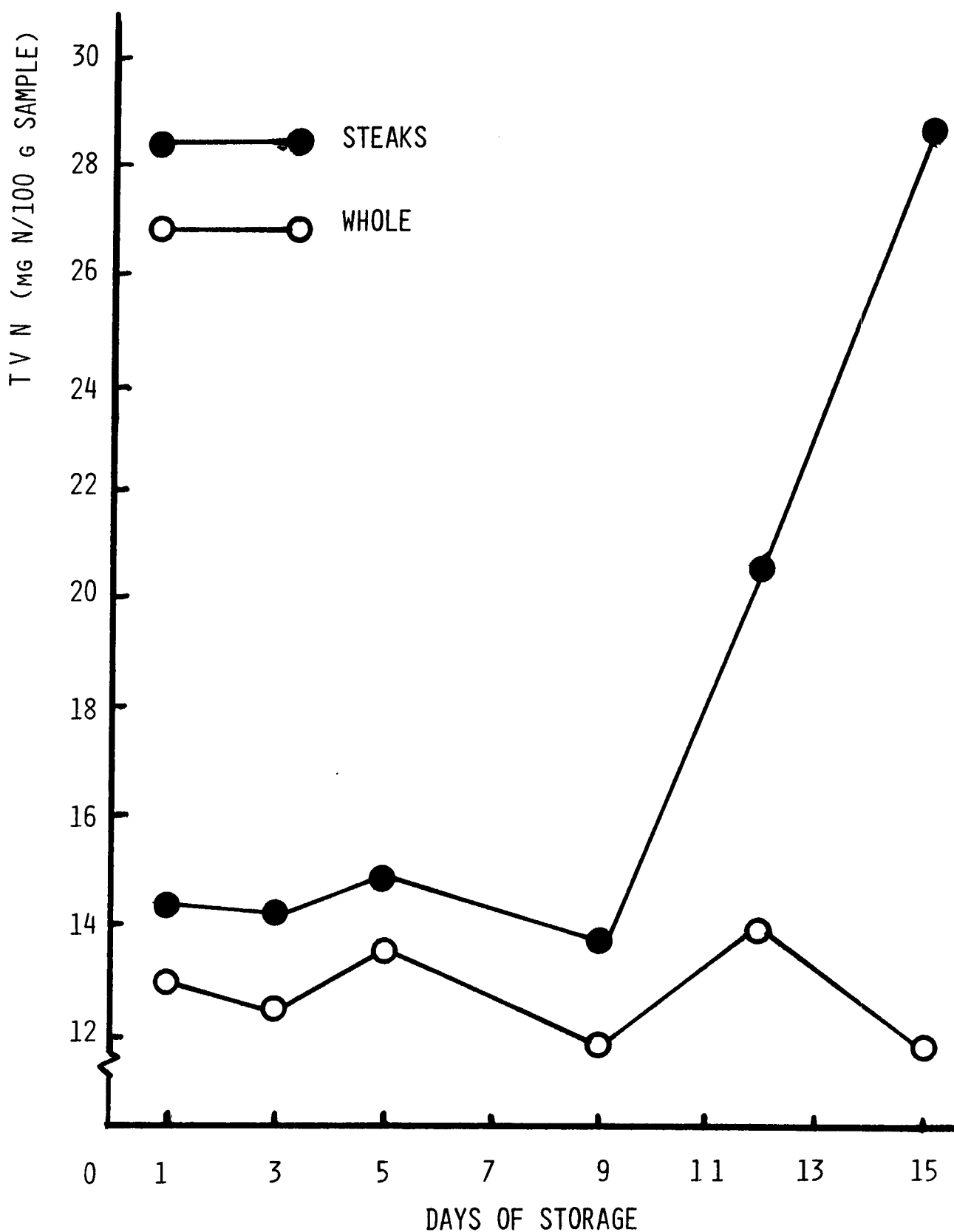FIGURE 3: TOTAL VOLATILE NITROGEN CONTENT OF SPANISH MACKEREL DURING STORAGE AT 4°C.

FIGURE 4: TOTAL VOLATILE NITROGEN CONTENT OF
KING MACKEREL DURING STORAGE AT 4°C.

values for skin-on fillets, it is recommended that fillets be
marketed skinless unless they are preferably marketed with the
skin-on.

Figures 5, 6, 7, and 8 show the TMA-N content of various
forms of croaker, white trout, Spanish mackerel and king mackerel
during refrigerated storage. These figures show that a significant
increase in TMA-N occurred before the TVN increased in fillets and
H&G fish in all species evaluated except king mackerel. The TMA-N
in king mackerel steaks increased at the same time as the TVN.
These results indicate that the TMA-N test may be a better indicator
of the incipient of spoilage than TVN because a significant increase
occurred at the same time the panel reported slight spoilage odors.

Although the data is not shown, TBA values for croaker and
king mackerel generally followed the same pattern as the TVN and
TMA-N values. TBA values for white trout and Spanish mackerel were
somewhat erratic, but generally increased until the panel judged
the products of borderline quality, then the values decreased. A
decrease in TBA values toward the end of refrigerated storage for
several species has been reported in the literature. Botta and
Richards (2), Castell, et al. (4), and Buttkus (3) experienced
similar results with salmon, cod, and trout. They attempted to
explain the results by suggesting that the oxidation products
(aldehydes, acids, etc.) may have reacted with the protein or some
other component of the muscle in a manner that protected the double
bond against oxidation. Rancid odors were not reported by the sensory
panel even at the end of storage.

Results of the microbial and pH analyses of all species and
product forms studied showed a progressive increase in values during
storage supporting results of the chemical and organoleptic evaluations.

Organoleptic assessments indicated that the shelf life of
Spanish mackerel and croaker fillets was 5 days; H&G fish 7 days;
and whole fish 10 days. The maximum shelf life of king mackerel
steaks was judged to be 9 days and whole fish 12 days. White trout
fillets had a shelf life of 6 days; H&G fish 8 days; and whole fish
about 10 days. It should be reemphasized that croaker, white trout,
and king mackerel were caught and held on ice 3 days before process-
ing into the various product forms. The Spanish mackerel were caught
1 day before processing.

CONCLUSIONS

Chemical and microbial results correlated well with organoleptic
evaluatons in determining the refrigerated shelf life of croaker,
white trout, Spanish mackerel, and king mackerel. The Conway
microdiffusion technique for determining TVN and TMA-N is most useful
in detecting spoilage in its early stages. TVN values above 16 mg
of N/100 g of sample is indicative of advanced stages of spoilage
and was confirmed by sensory panel evaluations. TMA-N values increased
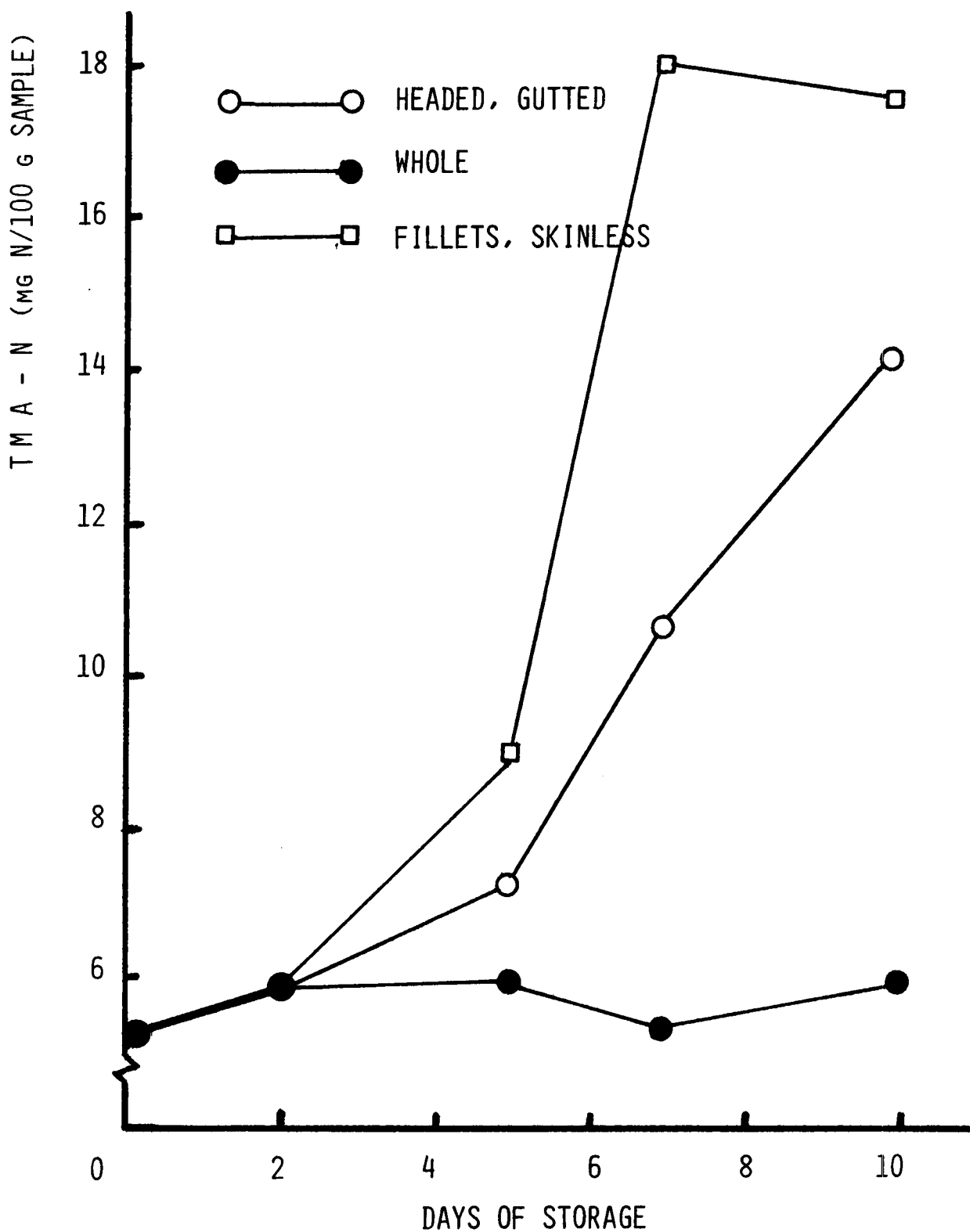significantly before the TVN values and may be a better indicator of

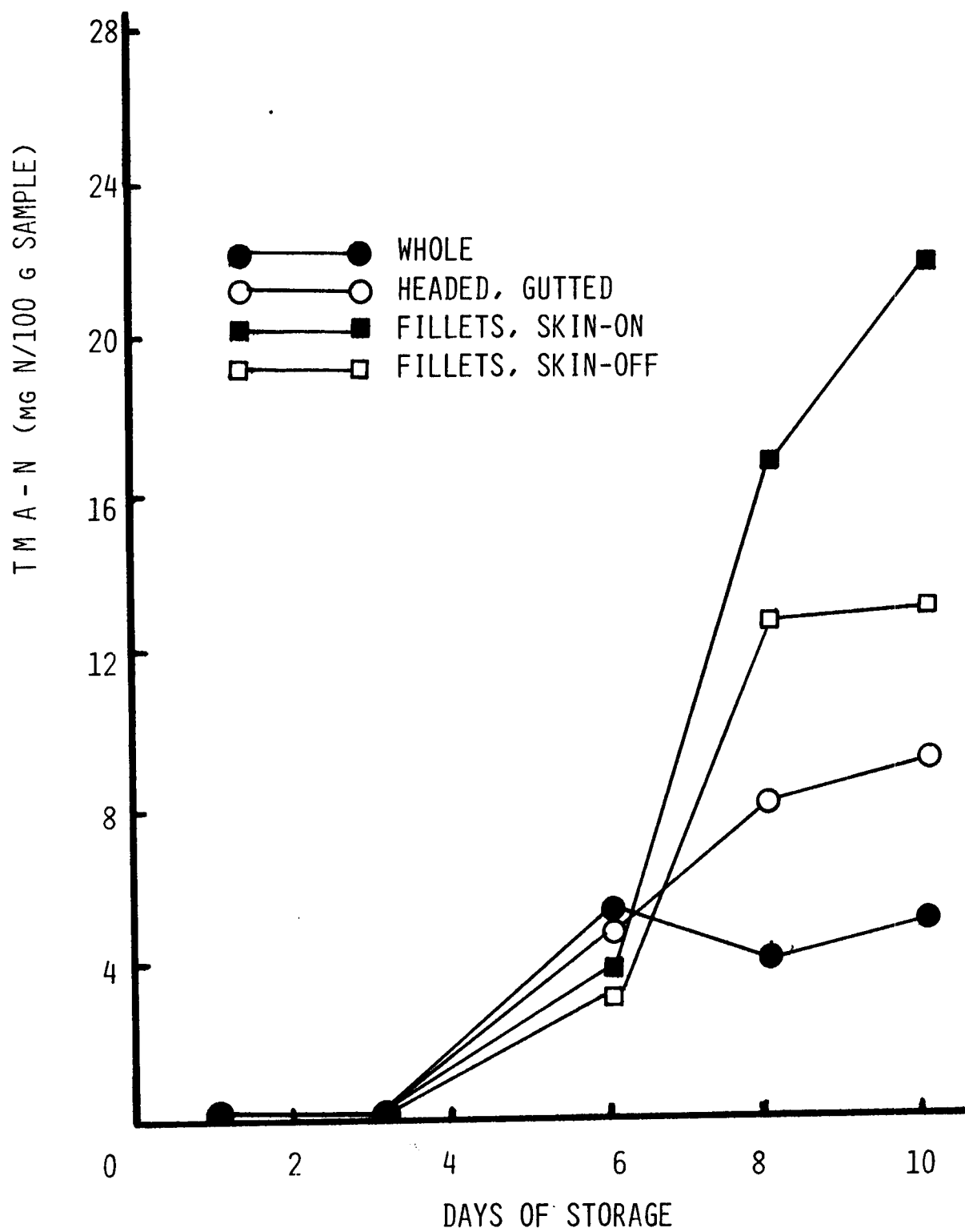FIGURE 5: TRIMETHYLAMINE-NITROGEN CONTENT OF CROAKER DURING STORAGE AT 4°C.

FIGURE 6: TRIMETHYLAMINE-NITROGEN CONTENT OF WHITE TROUT DURING STORAGE AT 4°C.
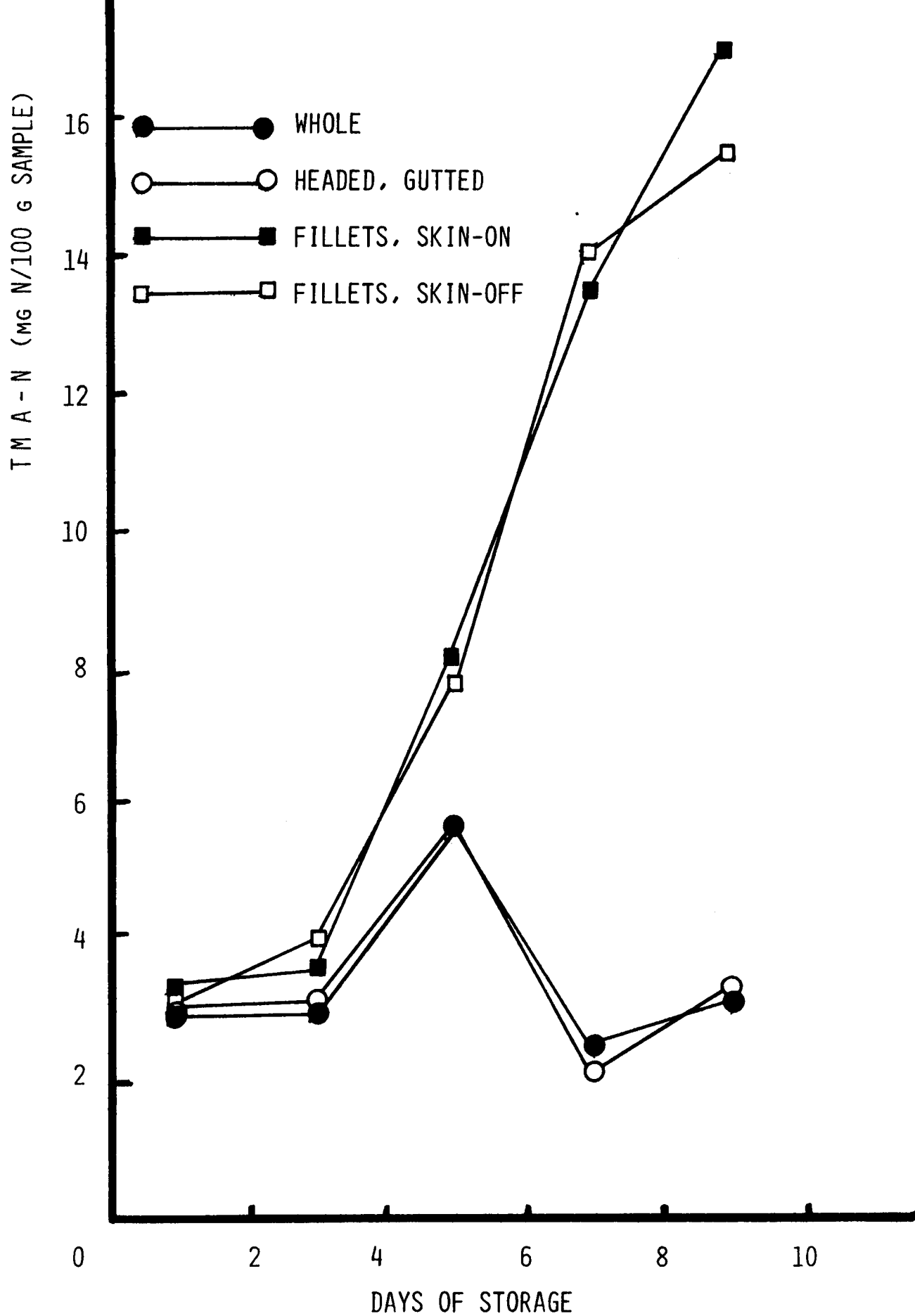
FIGURE 7: TRIMETHYLAMINE-NITROGEN CONTENT OF
SPANISH MACKEREL DURING STORAGE AT 4°C.

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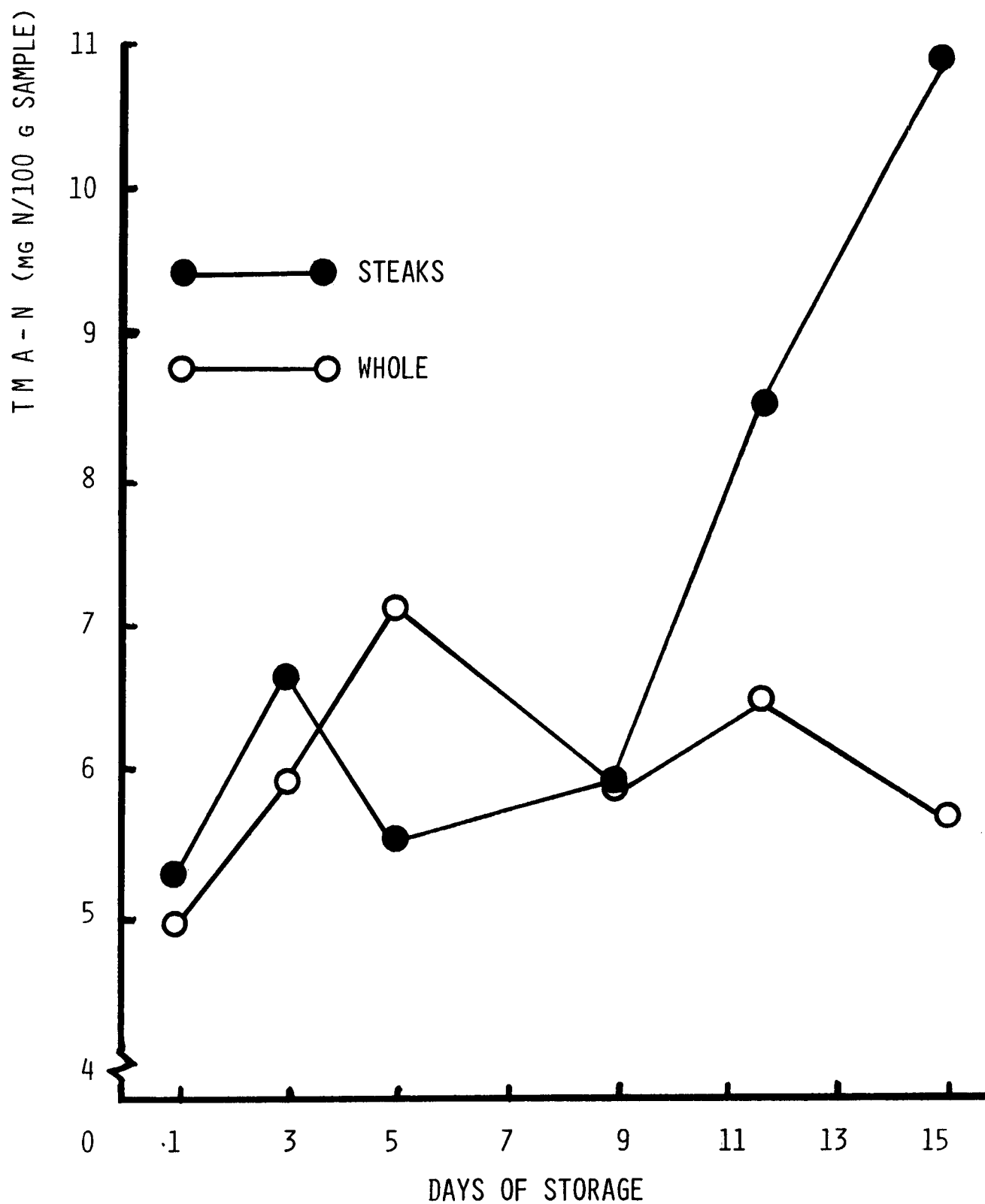
FIGURE 8: TRIMETHYLAMINE-NITROGEN CONTENT OF KING MACKEREL DURING STORAGE AT 4°C.

early stages of spoilage. TBA values for croaker and king mackerel increased with storage; values for white trout and Spanish mackerel increased during early storage but decreased at the end of storage. TBA values never reached the point (5 mg of malonaldehyde/kg of sample) as considered by many research workers to be significant. Results of the pH and microbial analyses supported chemical and organoleptic evaluations. The results show that fillets have a shorter shelf life than H&G fish, and H&G fish spoil more readily than whole fish.

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COMPOSITION AND STORAGE STABILITY OF SPANISH MACKEREL
AND RELATED SPECIES

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The U.S. commercial landings for Spanish mackerel (Scomberomorus maculatus) set new records in 1976 for both volume and value (NMFS, 1977). Despite this fact, these species have not reached their commercial potential and may be considered underutilized. The major technological barrier to full utilization of the Scomberomorus species is a limited stability in frozen storage due to the development of oxidative rancidity.

The only extensive frozen storage study reported with Spanish mackerel was that of Farragut in 1972. He stated that Spanish mackerel, eviscerated and frozen whole in the normal commercial process, "...begin to show signs of rancidity within as little time as a 3-month period and are usually rejected by taste panels between the sixth and ninth month of storage." In phase I of his study, Spanish mackerel fillets were either dipped or injected with antioxidant solutions. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and the chelating agent ethylene diamine tetra-acetate (EDTA) were included in the various treatments. Peroxide values and free fatty acids were measured and organoleptic tests were performed. Results were variable, but the EDTA treated samples had superior flavor and texture scores after 9 months of frozen storage at -10°F (-23°C). Several different chemical forms of EDTA were tested in phase II of the study. The treatments with disodium EDTA and tetrasodium EDTA were judged to be the most effective in preserving the quality of the fillets. The Spanish mackerel remained in good condition for over 12 months when it was treated with a solution of tetrasodium EDTA and vacuum packaged.

Harvesting, processing and transportation have a critical effect on the ultimate frozen storage life of mackerel. Normal processing methods for finfish harvested from the Gulf of Mexico were outlined by Cox and Nickelson in 1976. They list both Spanish and king mackerel among the top 10 table fish species from the Gulf. Quality control laws in Florida require that fish be iced aboard the harvesting vessel but this is often not the case in other Gulf Coast States, and the quality of fish suffers. The authors call for the application of information available on other fish to the solution of problems of Gulf species for which very little technological information exists.

Although published data on the composition, processing and preservation technology of the Scomberomorus species is very limited, a much larger base of information exists on the Atlantic mackerel (Scomber scombrus). Dingle (1976) prepared an excellent bibliography and literature review on the technology of these mackerel species. It includes pertinent information published through the year 1974.

Stansby and Lemon (1941) published an extensive report on the handling of fresh mackerel and recommended changes from the traditional methods in use at that time. Fråser, Pitts and Dyer (1968) correlated the deterioration in flavor of iced mackerel with measurements of inosine monophosphate (IMP) and hypoxanthine and described a relatively simple test for the estimation of IMP content and fish quality.

Ke and co-workers at the Halifax Laboratory of the Fisheries and Marine Service of Canada have been working with Atlantic mackerel for several years and have published a number of recent papers dealing with preservation and oxidative rancidity. Ke, Nash and Ackman (1976) reported on quality preservation in frozen Atlantic mackerel. Fish of initial good quality could be stored satisfactorily for 4 months at -26°C. Vacuum packaging extended its storage life to more than 1 year at -26°C and to at least 6 months at -18°C. Lipid oxidation rates, as measured by peroxide value and TBA number, were much faster for the lipids contained in the mackerel skin than for the meat lipids during frozen storage (Ke et al. 1977). The activity of the unknown pro-oxidative substance(s) was detectable at storage temperatures above -40°C, but not below. Ke, Ackman and Nash (1975) proposed a rancidity index based on TBA number for relatively fresh mackerel and peroxide value for more rancid fish. They concluded from organoleptic test results that mackerel with a molar TBA number of less than 6 u-moles malonaldehyde per kg of fish tissue was of excellent quality. This equals 0.43 mg per kg in the more commonly used TBA units. A higher TBA number combined with a peroxide value of less than 2.0 indicated acceptable quality. A peroxide value in excess of 2.0 for the meat lipids or 12.0 for the skin lipids is an indicator of unacceptable quality.

Proximate compositions and carbohydrate and energy contents of Scomberomorus species were extracted from 13 references and summarized in a publication by Sidwell et al. (1974). Ousterhout (1960) published chemical composition data and physical measurements for a group of Spanish mackerel and reported an average fillet yield of 58.6%.

The lipids and fatty acid compositions of Atlantic mackerel were described in detail by Ackman and Eaton (1971). Gopakumar and Nair (1972) reported the fatty acid compositions of the dark and white meats of seer fish. This Scomberomorus species is of much commercial importance in India.

Additionål work with Spanish mackerel is needed to find practical methods for preventing the development of rancidity during frozen storage. The results of various studies with Scomber species could be applied to the problem. According to Stoloff, Pucochar and Crowther (1948), the frozen storage life of mackerel fillets was extended by dipping them in a solution containing carrageenin and several different chemical antioxidants. Liljemark (1964) found that either dipping in 2% ascorbic acid solution or vacuum packaging would improve quality retention in mackerel fillets about as much as reducing the storage temperature from -20°C to -30°C. Bauernfeind et al. (1948) also retarded rancidity development in mackerel fillets by dipping them in an ascorbic acid solution and wrapping them in cellophane or Pliofilm.

The remainder of this paper describes a frozen storage study with Spanish mackerel that we are conducting and gives some preliminary results and conclusions. The purpose of the study was to measure changes in flavor and texture occurring in Spanish mackerel during frozen storage and to determine the protective effects, if any, of treatments with solutions of ascorbic acid (AA) and/or carboxymethyl cellulose (CMC).

MATERIAL AND METHODS

Spanish mackerel, freshly landed in late September on the northern gulf coast of Florida, were processed at the plant of Raffield Fisheries and packaged and frozen within 24 hours of capture. Experimental samples of skinless fillets were dipped for 1 minute in either (i) a 2% AA solution, (ii) a 1.5% CMC solution or (iii) a combination of 2% AA and 1.5% CMC. Also prepared were an untreated fillet control, a headed and gutted (H&G) sample treated with the AA/CMC combination and an untreated H&G control. An additional set of skinless fillets was prepared for storage at -40°C and used as a taste panel standard in later organoleptic tests.

The samples were packaged in polyethylene pouches and held in ice temporarily until they were placed on trays and frozen overnight in a -20°F freezer. The frozen samples were packed in insulated boxes and returned directly by air to the College Park Laboratory where they were stored at temperature of -26°C (-15°F).

Quality changes have been followed primarily by taste panel tests for flavor and texture, the thiobarbituric acid (TBA) test for rancidity, and texture measurements with a Kramer shear press. Proximate composition, amino acid and fatty acid analyses were also determined.

RESULTS AND DISCUSSION

Selected data on the chemical composition of the Spanish mackerel samples are briefly summarized in Table 1. There was very little variation in protein (20.42% average) or in ash content. There were greater variations in total lipid contents of the various samples, but the overall average was 7.71% lipid as measured by a chloroform-methanol extraction. The amino acid pattern indicated a good balance of essential acids with 9.02% lysine and 2.8% methionine based on crude protein content.

Two fatty acids, 16:0 and 18:1W9, make up approximately 50% of the fatty acid content of the Spanish mackerel lipids. The polyunsaturate 22:6W3 accounts for another 15% of the total. The 40.2% total saturated fatty acids is definitely higher than that reported for Atlantic mackerel by Ackman and Eaton (1971). It is slightly lower, however, than the value reported for Scomberomorus guttatus by Gopakumar and Nair (1972).

The quality of the Spanish mackerel samples has been evaluated at zero time and after 1, 3 and 5 months of storage at -26°C. A final sample remains to be analyzed at 7 months. The TBA numbers for fillets through the first 5 months of storage are plotted in Figure 1. The values for the treated samples peaked at 3 months and then decreased. This pattern has been observed in many other systems and shows that the TBA test is a much more useful indicator of quality during the early stages of rancidity development than it is during latter stages.

Average organoleptic scores relative to the taste panel standard (equal to zero) are plotted for the headed and gutted samples in Figure 2. The treated sample was ranked superior to the control in both flavor and texture. The higher flavor scores at 5 months are questionable since an internal standard rated higher than the expected zero value. Flavor scores for the fillets showed a general downward trend during storage. The

sample dipped in a 2% ascorbic acid solution has been ranked highest in both flavor and texture through a 5-month storage period.

A Kramer shear press was used as an objective measurement of texture and the shear force readings are plotted in Figure 3. Taste panel texture scores are subject to both individual preferences (e.g., for firmer or softer texture) and to a probable bias related to flavor characteristics. Although it is probably coincidental, both flavor and texture rankings for the fillet samples were in exact inverse order to the measured shear pressure at zero time and after 5 months. Further exploration of this and other factors must await completion of the study.

CONCLUSION

Spanish mackerel are valued food fish and are gaining increasing acceptance in national markets. Processing and storage must be carefully controlled, however, to assure that consumers are not "turned off" by products which have developed rancid flavors. Although very little information has been published on the preservation technology of Scomberomorus species, the technical literature of Scomber mackerel species should be useful for the solution of processing and preservation problems.

Preliminary results of a frozen storage study with Spanish mackerel indicate that treatment of fillets with an ascorbic acid solution is beneficial to quality preservation, but the use of carboxymethylcellulose dips does not appear to be worthwhile. The study will be reported in more detail when it is completed.

ACKNOWLEDGMENT

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Table 1. Chemical composition summary for Spanish
         mackerel samples.

PROXIMATE ANALYSIS:
                          71.71% MOISTURE
                          20.42% PROTEIN
                           1.27% ASH
                           7.71% TOTAL LIPIDS

AMINO ACIDS: 9.02% LYSINE
                           2.80% METHIONINE
                           3.06% HISTIDINE

FATTY ACIDS: 40.20% SATURATED
                          30.30% MONOENES
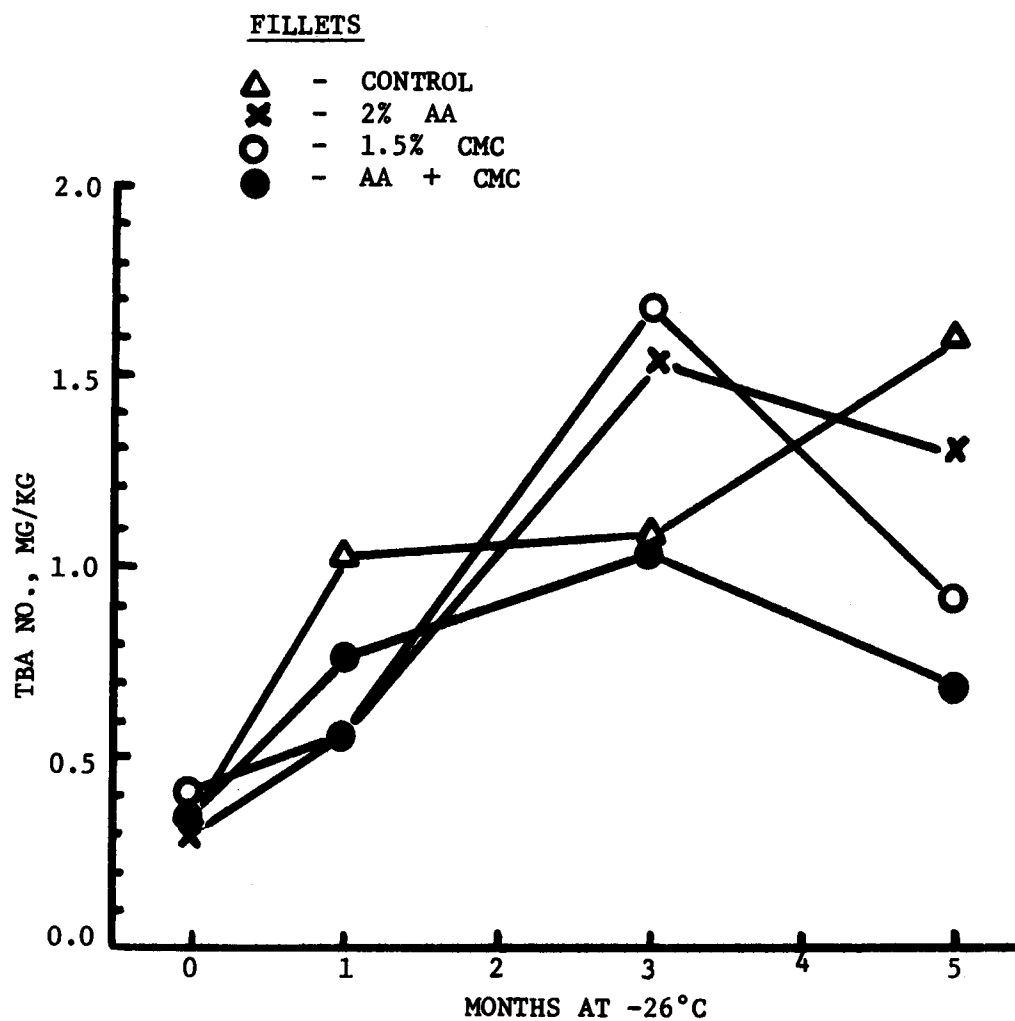   27.20% POLYUNSATURATED

Figure 1.   Trend of thiobarbituric acid (TBA) numbers for
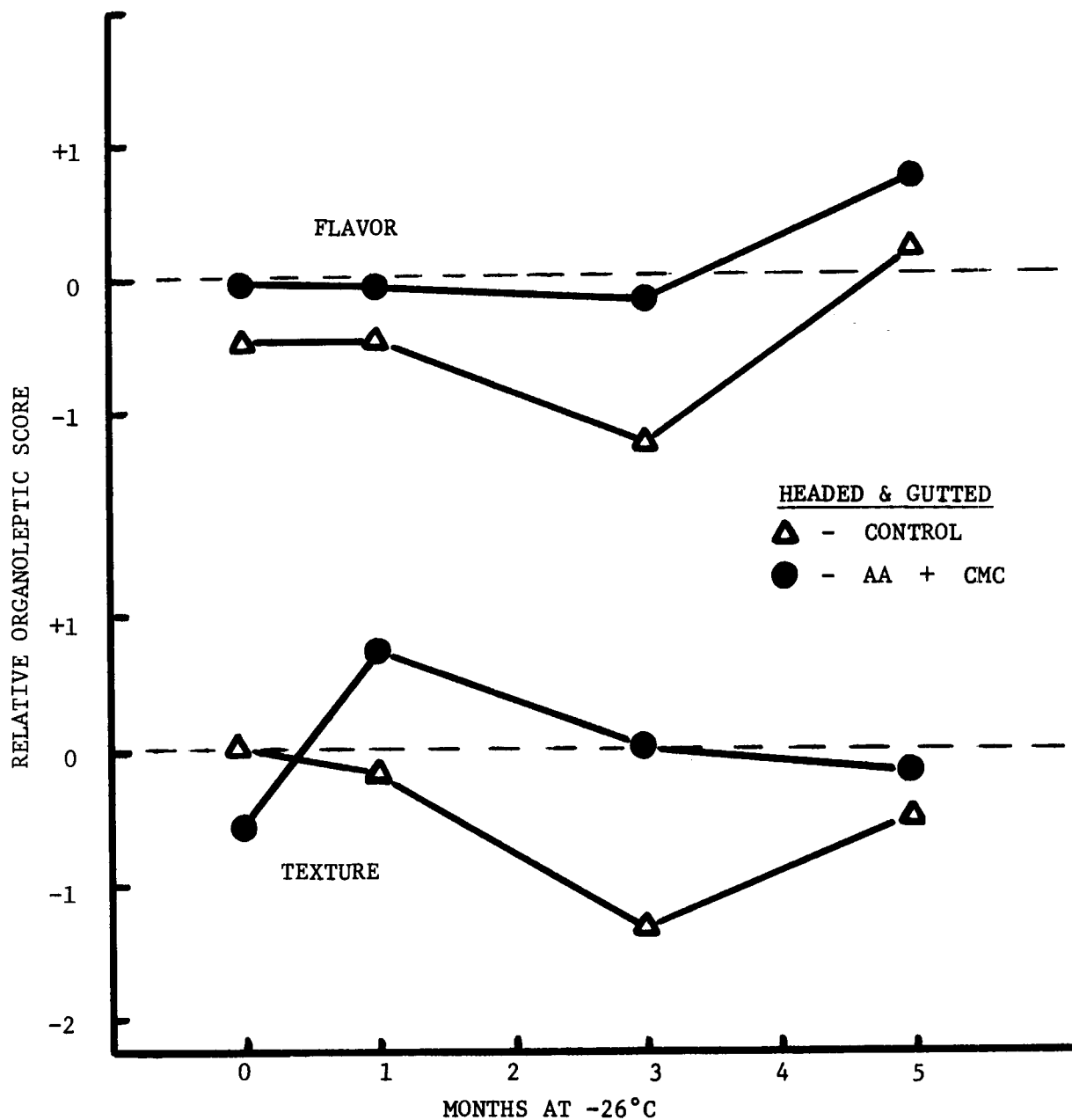mackerel fillets during frozen storage at -26°C.

Figure 2. Average organoleptic scores for headed and gutted
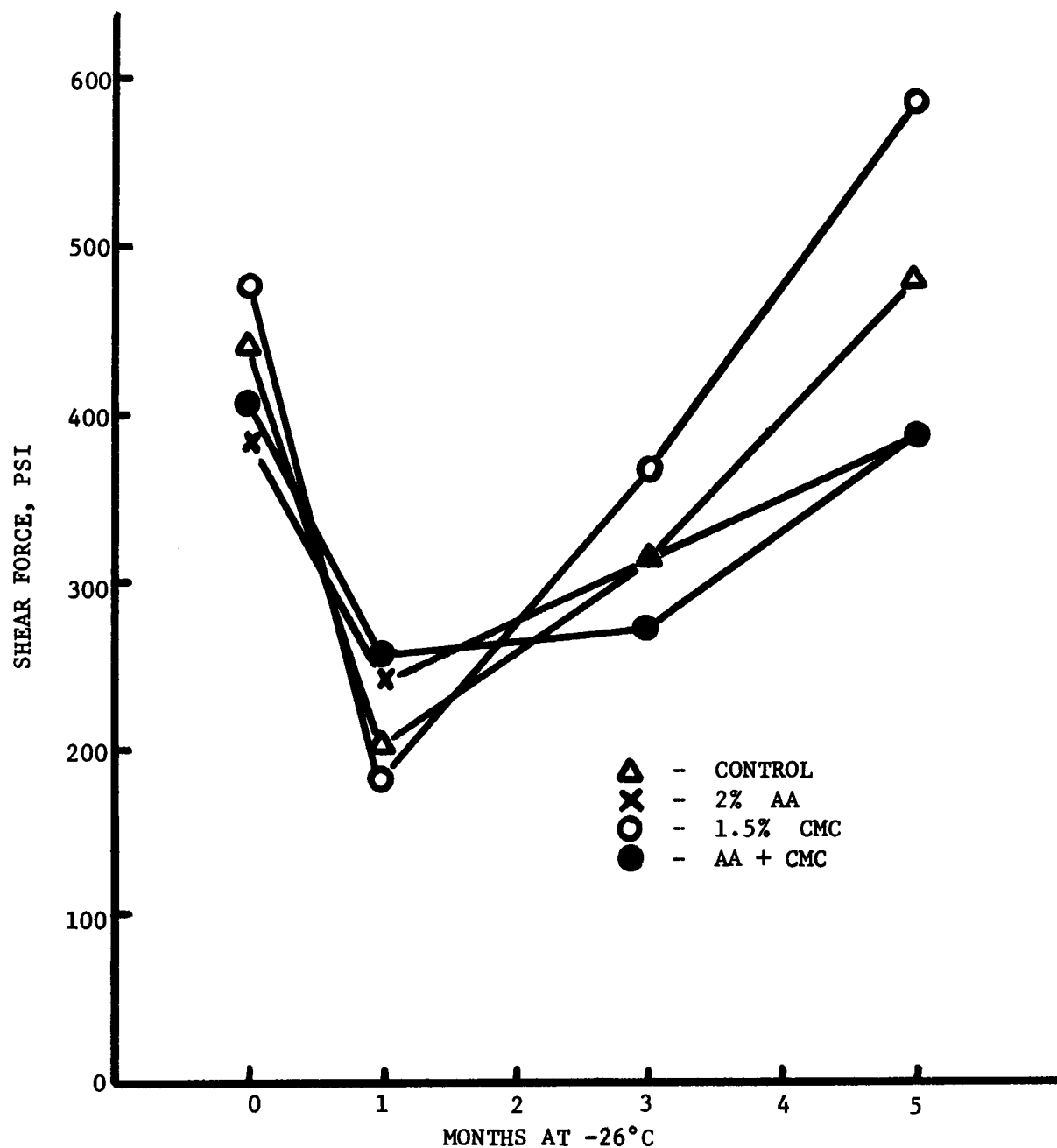Spanish mackerel stored at -26°C relative to standard
stored at -40°C.

Figure 3.   Texture measurements by Kramer shear press for Spanish mackerel fillets stored at -26°C.

COMPARISON OF TWO METHODS
FOR REMOVAL OF BREADING FROM
FROZEN RAW BREADED SHRIMP

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The FDA standard of identify for frozen raw breaded shrimp and the U.S. standard for grades of frozen raw breaded shrimp have established a minimum requirement of 50 percent shrimp material for frozen raw breaded shrimp manufactured and distributed in the United States.

The various state regulatory agencies have also established 50 percent shrimp material as a minimum requirement for this product.

Since an average of 50 percent shrimp material for lots of frozen raw breaded shrimp thus becomes a mandatory and legal requirement which the product must meet, it becomes necessary to perform an adequate number of determinations in order to know whether or not the product meets or exceeds the legal requirement. It is also necessary to provide an objective basis for the acceptance or rejection of production lots for the factor of percentage shrimp material. Since breading material is cheaper than shrimp, it is additionally important for the manufacturer to know how much shrimp he has in his product.

Because of the high volume of breaded shrimp produced in the United States, (approximately 100 million pounds annually), many determinations must be made by plant quality control personnel and government inspectors to provide this information.

It would be valuable, therefore, to have one official method which is rapid, reliable, repeatable and sufficiently simple for many different people with varying qualifications, experience and training to perform successfully and still provide acceptable dependable results which could be used to make decisions concerning the lots tested.

The purpose of the study presented in this paper is to compare two official methods presently being used in order to determine whether or not one method is superior to the other in measuring percent shrimp material.

This study also compares the time consumed by each method in making determination as well as the costs of each determination made by each method, which in addition to the legal requirement is of economic importance to industry and the government.

MATERIALS AND METHODS

Source and sample preparation

Commercially prepared frozen raw breaded shrimp packaged in institutional 2½ to 4 pound cartons were randomly sampled in a breaded shrimp manufacturing plant during normal production. Sampling was in accordance with USDC statistical sampling plan contained in "Regulations Governing Processed Fishery Products." Sample units contained specific count per pound, species and type. All sample units were regular breaded as opposed to lightly breaded. Domestic pink and brown shrimp were tested and their count per pound ranged from 12 to 38 shrimp per pound. Both fantail and round breaded shrimp were tested. Coarse and fine breading materials were in the sample. Each sample unit, however, contained the same style, type, species, count and coarseness of breading material.

Sixty sample units were randomly sampled and used to draw the subsamples for determination of percent shrimp material. Forty shrimp were randomly selected from each package. The forty shrimp were then randomly subdivided to yield the two 20 shrimp subsamples used to test the two methods.

The two subs of 20 shrimp were each tested by a different method conducted simultaneously by one operator. The tests consisted in removing the batter and breading material and determining the percentage of shrimp material using two different methods to remove the breading material. Hard frozen raw breaded shrimp were used for all determinations. Data for each pair of determinations were recorded on USDC score sheets.

Selection of methods

Method one employed the procedure commonly called the on-line method contained in the U.S. standard for grades of frozen raw breaded shrimp which normally is used on unfrozen raw breaded shrimp, and method two employed the procedure in the FDA standard of identity for frozen raw breaded shrimp, also found in the U.S. grade standard, and commonly called the end-product method.

Procedure

Method one hereinafter referred to in this paper as the, "soak and swirl method", utilized the following procedure:

Weigh the sample of 20 shrimp on a scale, determining the weight of the sample to the nearest 0.1 gram or 0.01 ounce. Place the sample in a water bath filled to three-fourths capacity and in a container maintained at 60° F. - 85° F.. After shrimp are submerged in water and breading becomes soft, a gentle swirling action with hands may be applied to the shrimp to speed up the removal of the breading. Stack a U.S. standard ½ inch mesh sieve with a diameter

of 12 inches over a U.S. standard sieve ASTM No. 20 with a
diameter of 12 inches and pour contents of the water bath con-
taining the shrimp into them. Remove the top sieve and drain on
an angle of 45 degrees for 2 minutes; then transfer the debreaded
shrimp to a balance. Rinse the contents of the No. 20 sieve into
a shallow pan and collect any particles of shrimp material (flesh,
tail fin etc.) and add to the shrimp on the balance and then weigh.

Calculate percent shrimp material:

Percent shrimp material

$$= \frac{\text{weight of debreaded sample}}{\text{weight of sample}} \times 100 + 2$$

Method two hereinafter referred to in this paper as the "wooden
paddle, machine stirred method", utilizes the following procedure:

Weigh the twenty shrimp sample. Fill a two gallon container,
approximately 9 inches in diameter three-fourths full of water at
70° F. - 80° F.. Suspend the machine driven 120 r.p.m. two-vaned
wooden paddle, each vane measuring approximately 1 3/4 inches by
3 3/4 inches, in the container leaving a clearance of at least 5
inches below the paddle vanes, and adjust speed to 120 r.p.m. while
machine is running, add shrimp and stir for ten minutes. Stack a
U.S. standard sieve, ½ inch sieve opening, with a 12 inch diameter
over a U.S. standard sieve No. 20 with a 12 inch diameter, and pour
the contents of the container onto them. Set the sieves under a
faucet, preferably with spray attached, and rinse shrimp with no
rubbing of flesh, being careful to keep all rinsings over the sieves
and not having the stream of water hit the shrimp on the sieve
directly. Lay the shrimp out singly on the sieve as rinsed. In-
spect each shrimp and use a rubber-tipped rod and the spray to
remove the breading material that may remain on any of them, being
careful to avoid undue pressure or rubbing, and return each shrimp
to the sieve. Remove the top sieve and drain on a slope for 2
minutes, then remove the shrimp to a weighing pan. Rinse contents
of the No. 20 sieve into a flat pan and collect any particles other
than breading, (i.e., flesh and tail fins) and add to shrimp on
balance pan and weigh.

Calculate percent shrimp material:

Percent shrimp material

$$= \frac{\text{weight of debreaded sample}}{\text{weight of sample}} \times 100 + 2$$

Treatment of data

Observations were made on 60 samples consisting of 20 shrimp
tested by method one and 60 samples of 20 shrimp tested by method
two. Results were recorded in percentages of shrimp material to the
nearest tenth of a percent. The results were tested by hypotheses
about the difference between the means of the results of the two

methods using statistical measures of whether the difference between the means of the two samples tested by the two methods indicated they were from the same or from different populations. The assumption taken is that they were from the same population, however, realizing that the two differing method techniques could not be applied to the same shrimp. Consequently, it became extremely important that the two 20 shrimp subs be as closely related as possible in regard to all manufacturing conditions, i.e., size, count etc.

The paired results were in most instances closely related. However, a few anomalies were observed which could indicate variance in the shrimp packed in the carton due to fill-ins for meeting net weight or count etc.

RESULTS AND DISCUSSION

Comparison of means

The mean of the soak and swirl method (method one) was 54.070 and the mean of the stir with wooden paddle method (method two) was 52.695 showing a difference of 1.375 for 60 observations. Table 1 shown below summarizes the results and the statistical data used in the computations.

N	$\bar{X}_1$	$\bar{X}_2$	VAR_1	VAR_2	SD_1	SD_2
60	54.070	52.695	25.3	18.5	5.03	4.30

TABLE 1
Statistical Comparison of Two Means

The Z score test was used to compare the two sample means of the percent shrimp material obtained by the two test methods. A Z score between -1.96 and +1.96 accepts the hypothesis that there is no difference at the 5% level of significance between the results of the two methods.

A null hypothesis with an alternative hypothesis was established as follows:

$$H_0 : \bar{X}_1 = \bar{X}_2$$

$$H_1 : \bar{X}_1 \neq \bar{X}_2$$

Results were computed as follows:

$$Z = \dfrac{(\bar{X}_1 - \bar{X}_2)}{\sqrt{\dfrac{S_1^2}{N_1} + \dfrac{S_2^2}{N_2}}}$$

$$Z = \dfrac{(54.070 - 52.695)}{\sqrt{\dfrac{25.30}{60} + \dfrac{18.5}{60}}}$$

$$Z = 1.61$$

Since Z falls in the acceptance region, (which for this test extends from -1.96 to +1.96), we accept the hypothesis that there is no difference at the 5% level of significance between the means of the results of the two methods, that the population is the same and that the two methods give statistically equivalent results for determining percent shrimp material on frozen raw breaded shrimp.

The mean of the soak and swirl method, (method one), was observed to be slightly higher than the mean of the stir with wooden paddle method (method two).

This suggests that some of the shrimp material may be lost by subjecting the shrimp to the chopping action of the wooden paddles, and the fine particles of shrimp material may be lost in the breading material left on the No. 20 sieve.

Comparison of time
Average time for running one sample by the soak and swirl method, (method one) for 57 observations was 5.16 minutes per observation.

Average time for running one sample by the stir with wooden paddle machine method, (method two) for 57 observations was 13.54 minutes. Method two, stir with wooden paddle machine, took 2.62 times as long as the soak and swirl method, (method one). Table 2 illustrates the comparison of time in minutes of the two methods in making one average observation.

N	$\sum T_1$	$\sum T_2$	$\bar{X}\ T_1$	$\bar{X}\ T_2$	DIF
57	294	772	5.16	13.54	8.38

TABLE 2
Time Required for Performance of Tests
(In Minutes)

Method two (stir with wooden paddle machine) required 2.62 times the number of minutes required by method one (soak and swirl).

Comparison of costs

Using the current hourly cost of USDC product contract inspection, which is $17.20 per hour, it was found that the stir with wooden paddle machine method, (method two), cost $3.89 per determination while the soak and swirl method, (method one), cost $1.48 per determination. This was a difference of $2.41, favoring the soak and swirl method, (method one). This cost comparison would vary with operations. The cost comparisons are shown in Table 3.

@ $17.20/Hr.	Ave. Time Per Unit	Cost Per Unit
Stir with Wooden Paddle Machine (Method Two)	13.54 min.	$3.89
Soak and Swirl (Method One)	5.16 min.	$1.48
Difference	8.38 min.	$2.41

TABLE 3
Average Cost for One Determination
Under USDC Inspection Rate

CONCLUSIONS

Numerical results of method one for removing the batter and breading materials from frozen raw breaded shrimp samples by soaking the shrimp in a water bath and swirling the shrimp gently by hand compared closely with numerical results of method two contained in the FDA standard of identity which consists of stirring the shrimp with a wooden paddle driven by a machine at 120 r.p.m. for ten minutes.

The latter method was time consuming, cumbersome and in many instances did not by itself remove all of the batter from the shrimp. It was required to remove the remaining material by rinsing the shrimp under a faucet and rubbing the shrimp with a rubber-tipped stirring rod. This increases the time of running the sample when using the FDA method and increases the chances of operator error and loss of shrimp material.

The fact that the mean of the soak and swirl method is slightly higher in percentage shrimp material than the mean of the machine, wooden paddle method suggests that perhaps some shrimp is lost using the machine, wooden paddle method, which suggests this method may not be as accurate as the soak and swirl method, (method one) referred to in the U.S. grade standard as the on-line method. The soak and swirl on-line method, therefore, appears to be superior to the FDA method (stir with wooden paddle machine).

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2. NATIONAL MARINE FISHERIES SERVICE. Recodified 1977. Code of federal regulations. Title 50 - chapter II. Subchapter G. Part 265. Subpart B - United States standards for grades of frozen raw breaded shrimp. Sec. 265.171 (v).

THE EFFECTS OF DIETARY FIBER ON SURVIVAL, GROWTH
AND FEED EFFICIENCY OF JUVENILE BLUE CRABS (<u>Callinectes sapidus</u>) 1/

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INTRODUCTION

Dietary fiber has long been recognized as an important component in the food supply of ruminant species (cattle, sheep, etc.). More recently, nutritionists, dieticians and medical experts have placed greater significance on dietary fiber's potential impact on the health and physiological well-being of man. Despite such attention, many questions need to be answered before the true effects of food fiber can be assessed in a consuming organism. Issues such as: 1) differences and similarities of natural vs. processed fiber, 2) fiber chelation of minerals in the intestine (Zn, Ca, Fe), and 3) decreased rate of uptake of nutrients, are considered in a series of papers presented during an international symposium on the subject of "Food and Fibre" (3). With the existing uncertainties as to the exact role(s) of fiber in the nutrition and health of terrestrial species, it is not surprising to find that little is known on this subject for crustaceans. However, according to Biddle (2) it has been a common practice to use non-nutritive fiber (cellulose) as a filler in experimental crustacean rations at levels ranging from 0% to 75% of the total dietary dry matter. Beyond creating unique processing problems as well as altering diet density (bulk), inclusion of such wide ranges of fiber in test feeds could play as much of a role in the final outcome of a study as the specific nutrient(s) under investigation. Therefore, our study evaluates the effects of variable levels of dietary fiber on the survival, growth, and feed efficiency of juvenile blue crabs maintained in the laboratory.

MATERIALS AND METHODS

Experimental Animals and Holding System

Wild juvenile blue crabs of both sexes were captured using an 18.2 m x 1.2 m nylon seine net with a mesh size of 0.6 cm, from Assawoman Bay, Delaware. Water temperature and salinity conditions were 26.5°C and 16.5 parts per thousand (o/oo), respectively, as determined with a temperature-salinity meter. The crabs were placed in styrofoam coolers containing dampened burlap to prevent dehydration and reduce aggression

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1/ Contribution Number 78-35C.

during transit from the capture location. Upon arrival at our laboratory the crabs were introduced into a recirculating artificial seawater (Instant Ocean) system maintained at a temperature of 25°C ± 1 and a salinity of 16 °/oo.

The system was composed of three, 3.2 m long x 0.4 m wide x 0.2 m deep "Gel-coated," fiberglass trays 2/, each equipped with a 5 cm (inner diameter) stand pipe and drain which emptied water into a series of mechanical and biological filters established in 75 L rectangular, poly-ethylene containers. A large 900 L reservoir received the filtered water and with the aid of a submersible pump (Little Giant, Model 4E-34NR) and polyvinyl chloride ducts, water was transferred to the animal trays. A polyethylene divider was placed perpendicular to the bottom, lengthwise in the center of each tray. Cross partitions were then secured between the two inner sides of each tray and the central divider, thus creating two rows of individual cubicles (18 cm long x 13 cm wide x 15 cm deep), 20 cubicles/row. The cross members were raised 0.3 cm above each tray's bottom to permit a constant unidirectional flow of water at a depth of 7 cm and to facilitate removal of fecal detritus and uningested feed residues. A hinged, transparent polystyrene top was secured to each of the holding trays. Room lighting was provided by conventional, fluorescent ceiling lamps connected to a timer set for a photoperiod of 13 and 11 hours of light and dark, respectively. Light intensity at the surface of the water in each tray was constant at 65 foot candles.

After a 1-week acclimation period the crabs were individually weighed, measured, and then assigned to the three dietary treatments (40 animals/diet) described below. Care was taken to equalize the initial biomass for all treatments. During the 9-week study, survival, molting character-istics, and feed offered were recorded. To assess the difference in growth and feed conversions between small and large animals, the 20 smallest crabs in each treatment group were placed in one of the two rows of cubicles in each tray (described above) and the largest animals in the second row of 20 cubicles (A and B groups). As the animals molted, new body measurements were recorded on a 5-day post-molt basis. Temperature and dissolved oxygen were measured daily. Other water quality parameters (ammonia, nitrite) were also carefully monitored. Additionally, 25% of the total water was replaced weekly.

Experimental Diets

Isonitrogenous diets were formulated (Table 1) to contain 3, 9, or 27% fiber. To achieve these levels, appropriate quantities of dietary starch were replaced with Solka Floc3/ cellulose. Menhaden fish meal and soy proteinate were the two major sources of protein in the diets and crude menhaden body oil provided the essential lipids/fatty acids. The

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2/ Fiberglass trays were fabricated by the Moorman Manufacturing Co., Gloucester, Va.

3/ Brand name product available from the Brown Co., Berlin, N.H. The use of trade names is merely to facilitate descriptions; no endorsement is implied.

oil also served as an excellent carrier for other dietary additives:
dl-α-tocopherol acetate, Santoquin4/, and crystalline β-carotene
(See Footnote 3, Table 1). Gelatin and sodium alginate were added to the
basal ingredients of each diet to enhance binding and water stability of
the pelleted feeds. The choline·Cl and vitamin premixes were modifications
of those used by Miller et al. (9). Each diet was processed in a similar
fashion. Except for the gelatin, all ingredients were blended in a
mixer for 20 minutes. The gelatin was dissolved in boiling water (1 part
water to every 5 parts of dry diet) and then added to the dry blend and
vigorously mixed for 4 to 5 minutes. The moistened product was then
pelleted, allowed to air dry for 30 minutes, placed in a forced-air oven
for 15 minutes at 100°C, cooled, sampled for proximate analyses, and frozen
for later feeding. Using the above procedures, pelleted feeds were obtained
with an average length of 2 cm and a diameter of 0.5 cm.

The experimental diets were fed during a 5 to 6 hour feeding period,
6 days a week. Rather than place excess quantities of feed in each animal
cubicle once-a-day, smaller quantities were offered up to a maximum of
three times during each feeding period according to the animal's appetite,
thus reducing feeding wastage.

Chemical and Statistical Analyses
Samples of each diet were analyzed for dry matter, crude protein, ether
fat, ash, and phosphorus, using AOAC (1) procedures. Total fat and calcium
were determined according to the methods of Smith, Ambrose, and Knobl (10),
and Kingsley and Robnett (7), respectively. Where applicable, experimental
data were subjected to a one-way analysis of variance (11) to determine
statistical differences between treatments.

RESULTS AND DISCUSSION

Results from the chemical analyses (Table 2) of the experimental
diets were in agreement with anticipated nutrient profiles based on the
proportions of individual ingredients incorporated into such diets. The
high fiber (27%) diet was more difficult to pellet and had poorer water
stability than the lower fiber diets. Even with the recently acquired cap-
abilities provided by cooker-extrusion processing, we experienced difficulty
in preparing high fiber diets. Besides processing related problems, one
of the drawbacks to the inclusion of high fiber in crustacean diets is that
high quality dietary energy sources are replaced with a non-nutritive
material from which only limited energy can be assimilated by the organism.

Overall survival (Table 3) equaled 87 and 95% for juvenile blue crabs
fed the 3 and 9% fiber diets, but only 67% for those fed the 27% fiber diet.
Most deaths in the group fed the 27% fiber diet, occurred in the A group
between the 6th and 9th weeks of the experiment (See survival data in Table
4). Before death, there were characteristic changes in the animal's appearance
and activity: darkened and/or retracted eyestalks, discoloration of the
carapace, extension of the chelae, occasional disorientation of body
equilibrium and lack of feed response. Gross internal changes were a chalky

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4/ Brand name for the anti-oxidant, ethoxyquin; which is available from
the Monsanto Co., St. Louis, Mo.

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white color of the body fluid(hemolymph) with a lack of normal clotting and a creamy colored hepatopancreas with very little texture. Histopathological examinations[5] on various tissue samples from several of the dead crabs revealed a Herpes-like viral infection. Interestingly, this same virus was also detected in normal crabs captured from the same area as the experimental animals. Conceivably, this virus may act on the crab when it is subjected to stress conditions. Although highly speculative, the closed system confinement plus diet related factors may have accentuated the development of the Herpes-like infection and subsequent deaths among the A group assigned to the 27% fiber diet.

The biomass increase among the A groups receiving the 3 and 9% fiber diets was similar (73.8 and 74.4 g, respectively) while the heavy mortality in the 27% fiber A group negated any comparison with the lower fiber treatments (Table 3). Although inconclusive, there was an apparent reduction in overall growth (biomass increase) in the B group fed the 27% fiber diet (Table 3). No treatment effects (P > 0.05) could be detected for percent post-molt weight increases (computed only for those crabs that survived the entire 9-week experiment); however, it is noteworthy that such increases were somewhat greater than the 30 to 33% estimates normally reported (12) for the blue crab in its natural habitat. When the growth of these same animals was expressed as a percent increase of final over initial wet body weight (Figure 1), slight but consistently lower values were determined with each increase in dietary fiber level for both small and large animals. The more rapid growth rates among the small animals within each dietary treatment are also shown in Figure 1.

Food conversion ratios (total feed offered/total biomass increase) computed for each treatment at 3, 6, and 9 weeks are presented in Table 4. We observed a consistently more aggressive feeding response among crabs fed the 27% fiber diet than that of the animals assigned to the lower fiber treatments. With the exception of the better conversion values for the large animals in each treatment, no distinct trends could be determined after the 3rd week. Generally, the efficiency of feed utilization in a young, actively growing animal is superior to that of the older animal within the same species; thus, the inferior conversion ratios among the A groups within each treatment were unexpected. Factors that could have contributed to this response include the feeding technique employed, size and water stability of the pelleted feeds, the ingredients used and their bioavailability in conjunction with physiological limitations, such as differential development of digestive capacities between young and older animals.

By the 6th week of the experiment, food conversion rations for both the A and B groups assigned the higher fiber diet were noticeably inferior to those from the lower fiber diets (Table 4). This pattern was repeated among the B group animals when the cumulative 9-week conversion ratios were computed. Without doubt, feed conversions were influenced by overall survi-

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[5] Dr. Phyllis Johnson, Crustacean Pathobiologist, Northeast Fisheries Center/Oxford Laboratory, Oxford, MD 21654.

val in the three treatments. However, except for the A group assigned the 27% fiber diet, we believe that the trends presented in Table 4 are indicative of the potentially negative impact of excessive fiber in the crustacean's diet.

There are various ways fiber could exert such an influence. For instance, by using high levels of fiber one is effectively diluting valuable dietary nutrients with a non-nutritive material that has been estimated by Forster and Gabbott (4) to be poorly digested/assimilated (20%) by the prawn (Palaemon serratus). Also, the relative length of the crustacean's alimentary tract is considerably short; therefore, under normal feeding conditions, passage rates through the gut are quite rapid. In terrestrial animals, including man, ingestion of high fiber diets increases passage rates, and although the exact mechanisms are not well-defined as pointed out by Hegsted (6), it is assumed that an acceleration in passage rates would also occur in the crustacean when dietary fiber levels are increased. With decreased time spent in the gut, overall digestion and assimilation of ingested nutrients in all likelihood is markedly reduced. Such an effect on nutrient digestibility was clearly demonstrated in a report by Garrison et al. (5) when laboratory rats were fed diets containing 5, 10 or 15% "acid-detergent-fiber." Similar impairments in overall nutrient digestibility have also been reported (8) in warm water species such as the channel catfish (Ictalurus punctatus) when fed diets containing high levels of fiber.

Whereas excess non-nutritive fiber (cellulose) may exert a negative effect on overall nutrient assimilation and growth in crustaceans, limited quantities of raw or natural food fiber may be beneficial as suggested by the data of Venkataramiah et al. (14). When shrimp (Penaeus aztecus) were fed up to 5.5% vegetable fiber in their diet (provided by dehydrated, finely ground turnip greens), improvements were observed in survival, growth, and food conversion efficiencies. Therefore, the source or type of dietary fiber is an important consideration. Similar observations have been reported by Van Soest and Robertson (13).

CONCLUSION

Although the results obtained during our research are based on limited observations, we conclude that high dietary fiber levels (27% of total dry diet) exert a negative impact on growth and food conversion efficiencies in juvenile blue crabs. This is sufficient reason to caution against the indiscriminate use of highly variable levels of fiber as a dietary filler. In the ultimate development of cost-effective, nutritionally balanced crustacean feeds, total dietary fiber will be an important consideration. Thus, there is a need for experiments to further elucidate the significance of dietary fiber in crustacean nutrition and to also conduct studies on the physiological impact on rates of passage and nutrient digestibility and assimilation coefficients.

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Table 1. Experimental Diet Composition (%).1/

Ingredients	Percent Dietary Fiber 3	9	27
Menhaden Fish Meal	34.48	34.48	34.48
Soy Proteinate	8.64	8.64	8.64
Instant Clear Jel (Starch)2/	34.58	28.58	10.58
Solka Floc Cellulose	3.00	9.00	27.00
Calcium Carbonate	2.00	2.00	2.00
Menhaden Oil Premix3/	5.03	5.03	5.03
Gelatin	4.00	4.00	4.00
Sodium Alginate	3.50	3.50	3.50
Glucosamine·HCl	3.00	3.00.	3.00
Choline·Cl Premix4/	0.80	0.80	0.80
Vitamin Premix5/	0.67	0.67	0.67
Cholesterol	0.30	0.30	0.30

1/ Expressed as percent of air dry weight.

2/ Modified food grade starch available from National Starch & Chemical Corp., Bridgewater, N.J.

3/ Oil premix provided 300 mg dl-α-tocopherol acetate, 200 mg ethoxyquin and 30 mg crystalline β-carotene per kilogram of air dry diet.

4/ Premix consisted of 75% Solka Floc cellulose and 25% choline·Cl.

5/ Vitamins premixed on Instant Clear Jel starch to fortify each kilogram of dry diet with 10,000 I.U. vitamin A; 2,000 I.U. vitamine D_2; 60 mg niacin; 60 mg ascorbic acid; 40 mg para amino benzoic acid; 30 mg calcium pantothenate; 10 mg thiamine·HCl; 10 mg pyridoxine·HCl; 10 mg riboflavin; 5 mg folic acid; 2 mg menadione; 400 ug biotin and 40 ug vitamin B_{12}.

Table 2. Proximate Analyses of Experimental Diets.[1]

Criteria	Percent Dietary Fiber		
	3	9	27
	(%)		
Crude Protein	35.8	35.9	35.8
Ether Fat	8.6	8.4	9.3
Total Fat	10.9	10.2	11.0
Total Ash	9.2	8.8	8.8
Calcium	2.7	2.5	2.6
Phosphorus	1.1	1.0	1.1
Total Fiber[2]	3.6	9.6	27.6

[1] Data expressed as percent of absolute dry matter.

[2] Fiber levels based on actual quantities of Solka Floc cellulose added to the diets, which also include 0.6% contributed from the choline·Cl premix (see footnote 4, Table 1).

Table 3. Overall survival, biomass increase, and post-molt weight changes of juvenile blue crabs fed diets containing different levels of fiber.

	Percent Dietary Fiber					
	3		9		27	
Criteria	A[1/]	B	A	B	A	B
Survival, %	85	90	90	100	50	85
Initial Biomass, gm	80.7	417.2	79.7	420.8	80.3	418.7
Final Biomass, gm	154.5	647.2	154.1	687.6	83.5	527.0
Biomass Increase, gm	73.8	230.1	74.4	266.8	3.2	108.3
Post-Molt Weight Increase, %[2/]	36±1.1	38±1.2	37±1.2	36±1.0	35±1.4	36±0.9

1/ Used to designate small (A) and large (B) animal groups within each treatment.

2/ Average post-molt weight increase ± Standard Error computed for only those animals in each treatment that survived the entire 9-week study.

Table 4. Feed conversion ratios of juvenile blue crabs fed diets containing different levels of fiber.

| | Percent Dietary Fiber | | | | | |
| | 3 | | 9 | | 27 | |
Criteria	A[1]	B	A	B	A	B
	(0 to 3 weeks)					
Survival, %	95	100	95	100	90	95
Feed Offered, gm	98.0	186.4	106.4	186.3	111.2	202.4
Biomass Increase, gm	26.9	146.5	44.6	132.3	27.6	140.0
Feed Conversion[2]	3.6	1.3	2.4	1.4	4.0	1.5
	(0 to 6 weeks)					
Survival, %	95	90	95	100	80	90
Feed Offered, gm	184.9	348.1	205.8	360.8	199.0	398.4
Biomass Increase, gm	53.4	174.6	59.0	201.6	45.1	97.6
Feed Conversion	3.5	2.0	3.5	1.8	4.4	4.1
	(0 to 9 weeks)					
Survival, %	85	90	90	100	50	85
Feed Offered, gm	273.1	504.6	303.9	531.5	272.4	558.2
Biomass Increase, gm	73.8	230.1	74.4	266.8	3.2	108.3
Feed Conversion	3.7	2.2	4.1	2.0	[3]	5.2

1/ Used to designate small (A) and large (B) animal groups in each treatment.

2/ Expressed as the ratio between food offered over biomass increase.

3/ Excessively high mortality during the last 2 weeks of the study negated any meaningful estimate of food conversion by this particular group of animals.
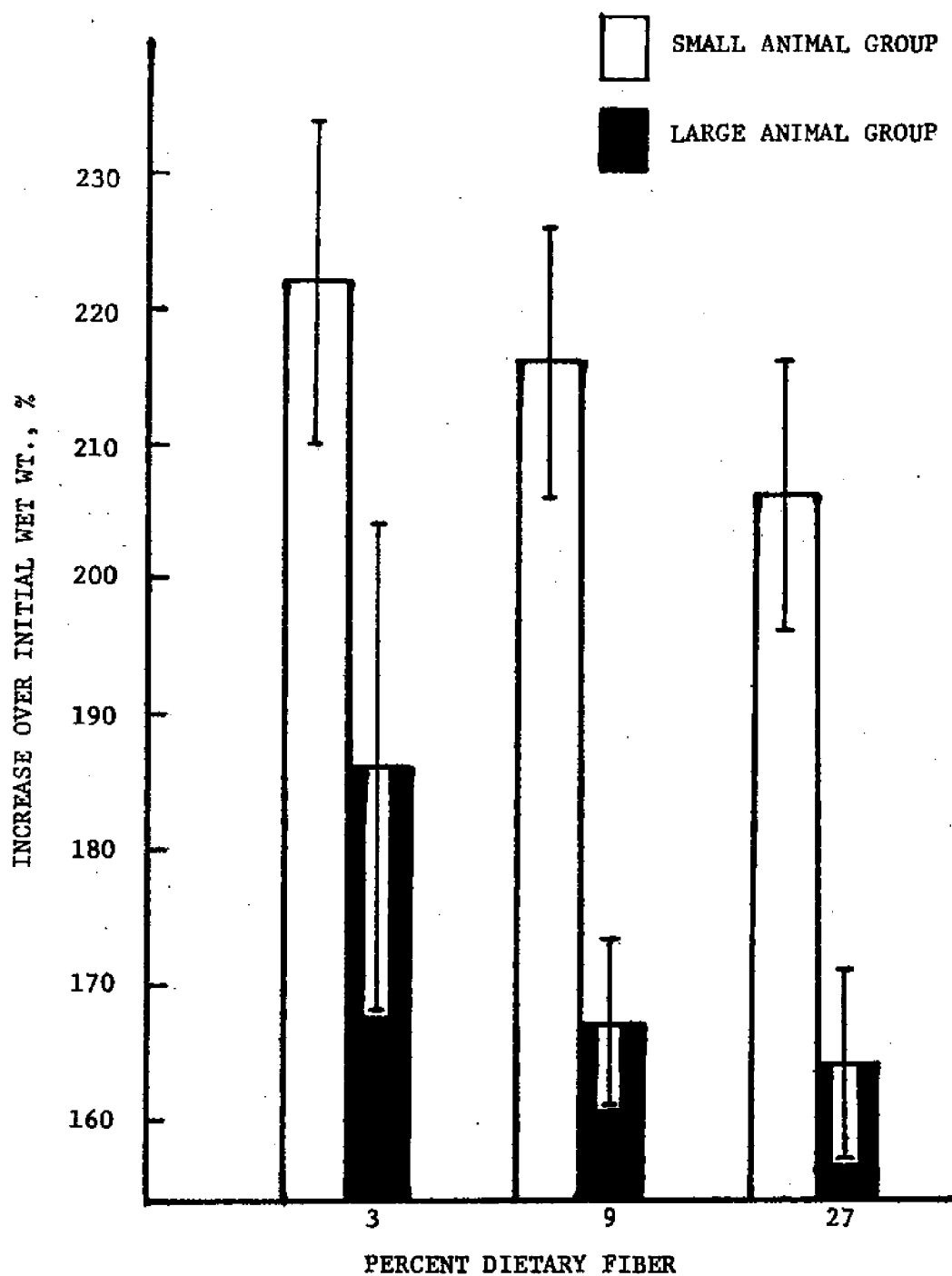
FIGURE 1. TOTAL PERCENT WEIGHT INCREASE OVER INITIAL
WET BODY WEIGHTS OF JUVENILE BLUE CRABS FED DIETS CON-
TAINING DIFFERENT LEVELS OF DIETARY FIBER (MEAN VALUES
± STANDARD ERROR OF THE MEANS).

EVALUATION OF SELECTED HOLDING SYSTEM ENVIRONMENTS USED IN LABORATORY STUDIES WITH JUVENILE Macrobrachium rosenbergii [1]

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INTRODUCTION

Before studying the nutritional requirements of juvenile prawn, the physical conditions of the holding system should be ideal to allow animal response to the specific dietary component under investigation. Besides water quality and biomass load, other system characteristics such as size of animal cubicles, the presence of substrate or intra-animal contact must be considered. Studies have shown that the use of artificial substrates and shelters can reduce cannibalism and allow higher stocking densities with several crustaceans including the blue crab (4), the common prawn (2), and the freshwater prawn (7,8). Goyert and Avault (3) reported better growth together with a shift from antagonistic to passive behavior in crayfish when stocked at higher densities in a recirculating culture system. Apparently this phenomenon does not occur in Machrobrachium based on a study by Sandifer and Smith (6), in which survival and growth varied inversely with population density. Light intensity also exerts an influence on crustacean behavior and performance as demonstrated by Sick et al. (9) and Nakamura (5).

Only when the impact of such factors becomes known can one truly assess the specific effects of nutritional parameters on the growth and development of cultured organisms. Therefore, our studies with juvenile Macrobrachium rosenbergii were to determine: (a) the effects of compartment size, substrate, and intra-animal contact on survival, growth, and molting behavior (Expt. I); and (b) the influence of two extremes in light intensity on survival and growth (Expt. II).

MATERIALS AND METHODS

Experiment I

A closed flow-through recirculating system consisting of three compartmented animal holding trays and a series of mechanical and biological filters (1) was structurally modified so that each tray contained 26 cubicles of four different sizes with and without protective substrates. Water within each tray was maintained at a constant depth of approximately 7 cm. The substrates had both horizontal and vertical components and were fabricated from polystyrene "egg-crate" and fine mesh plastic screening on a proportional basis to cubicle size. Both schematic and tabular data on the holding trays are shown in Figure 1 and Table 1.

Juvenile prawn with an average length of 2 to 3 cm were individually placed in the smallest cubicles and groups of four prawn were placed in all other compartments. Water temperature was maintained at 27°C ± 1 and other parameters of water quality carefully monitored. Approximately 25% of the system's water supply was replaced weekly. Prawn were fed a standard control ration (Table 2) at a daily rate equivalent to 5% of their total biomass. Feeding rates were adjusted at 3 week intervals and changes in survival, molting frequency, and growth were monitored over the 9-week study.

Experiment II

For the light intensity study, twelve, 56 liter (1) aquaria tanks were equipped with side mounted power filters and sub-gravel filters that covered one-half of the bottom surface of all tanks. An artificial substrate with both vertical and horizontal components was also placed in each tank. Contrasting light intensities were achieved by covering six tanks with clear polyethylene film and six tanks with black, opaque film. Light intensities measured at the water's surface were 45 and 2 foot candles for the light and dark tanks, respectively; a photoperiod of 8 hrs light and 16 hrs dark was employed.

Groups of 20 prawn were randomly selected, individually weighed (overall mean weight = 0.108g ± 0.007) and placed in the 56 l experimental tanks. Water quality parameters (temperature, dissolved oxygen, ammonia, and nitrite nitrogen) were closely monitored; 50% of the water in each tank was replaced weekly. Animals were fed a pelleted diet (Table 2) at 10% of their biomass daily. Survival, feeding rates, growth, and food conversion were determined at each 3-week interval during this 12-week study.

RESULTS AND DISCUSSION

Experiment I

With regards to survival, three points are illustrated in Figure 2. First, and most obvious, with each increase in compartment size animal survival increased, a pattern consistently repeated at each 3-week interval. Second, survival was further enhanced in the small (230 cm^2) and medium (460 cm^2) sized compartments when protective substrates were provided; whereas, in the large cubicles (689 cm^2) substrate availability had no apparent effect. Third, in the 9th week, survival in the small compartments with substrate was approximately 8% higher than that for prawn in medium sized cubicles without substrate. The enhanced survival resulting from the use of protective substrates together with the inverse relationship between survival and initial densities as shown in Table 1 are consistent with earlier studies on the effects of substrate (2,8) or density (6) on crustacean survival under conditions of close confinement. While these earlier reports addressed the singular effects of either substrate or density, the data represented in Figure 2 illustrate the combined influence of both variables on animal survival.

As with survival, molting frequency was affected by both compartment size (density) and substrate (Figure 3). With the exception of the 3rd week, the number of documented molts was noticeably greater among group reared prawn in the medium (460 cm^2) and large (689 cm^2) cubicles with

substrates. For the small (230 cm^2) compartments, the presence or absence of substrate had no consistent effect on molting behavior. When paired comparisons were made between small and medium and medium and large cubicles, molt frequencies were consistently higher in the smaller compartments with substrate than in the larger without substrate.

Unfortunately, the rather high mortality encountered in this study has negated any valid interpretation on the effects of compartment size and substrate on animal growth patterns. Based on very limited data (not shown), no differences could be detected between the growth of prawn placed in the various sized compartments in original groups of four and that of the individually maintained animals in the 115 cm^2 cubicles. A viable tagging method would have been most useful in monitoring live body weight changes among the group reared animals. Currently, several methods of animal identification are under consideration including the use of dyes, different types of tags, and external applications of water resistant materials (paints/epoxy resins).

Experiment II

Light-intensity study results are presented in Figures 4-6. Overall survival remained relatively high at both light intensities during the first 9 weeks of the study but decreased sharply thereafter (Figure 4). Although not significantly different ($P > 0.05$), survival of prawn maintained under reduced light intensity was consistently lower than that at the higher intensity. Nakamura reported (5) that the circadian rhythm of spontaneous locomotor activity in M. rosenbergii reaches a maximum during darkness. As such, the frequency of animal encounters would increase, thereby increasing the chances for cannibalism of newly molted animals. This behavioral pattern is consistent with the somewhat greater mortality observed in the low light-intensity treatment.

Mean body weights of prawn grown under the reduced light intensities were significantly heavier ($P \leq 0.05$) at the 12th week interval (Figure 5). Concomitant with the sharp decline in overall survival between weeks 9 and 12, there was a noticeable increase in apparent growth for both treatment groups. In all likelihood this was directly related to a greater rate of cannibalism during the last 3 weeks of the study.

Feed conversion ratios determined at each 3-week interval are presented in Figure 6. During the first 6 weeks of the study, conversions ranged from 2 to 1 to 3 to 1, becoming less efficient with each subsequent time interval. As with growth, the changes in feed conversion were most likely affected by the increased mortality among both treatments during the last 3 weeks of the experiment. Under the conditions of this study, light intensity had no significant effect ($P > 0.05$) on food conversion, although slightly better ratios were computed from the 6th week on for the low-intensity group.

CONCLUSIONS

Based on observed results, it is concluded that the relationship between available space and animal density was the primary factor in determining survival and molting activity of the prawn. The use of protective substrates provided greater advantages when used under less favorable space-density conditions where intra-animal contacts were more

frequent. Although inconclusive, results from the light-intensity study indicated somewhat lower survival but better growth and feed conversion among prawn maintained under decreased light intensity. Additional work is required to better quantitate the relationships between various system parameters (spatial allocations, substrate, light intensity/photoperiod) and prawn growth and performance to more accurately assess animal response to different dietary profiles.

ACKNOWLEDGMENTS

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TABLE 1. COMPARTMENT SIZE AND INITIAL ANIMAL DENSITY ASSIGNED TO EACH OF THREE HOLDING TRAYS IN A CLOSED, FLOW-THROUGH EXPERIMENTAL SYSTEM 1/

NUMBER OF COMPARTMENTS2/	DIMENSIONS LENGTH cm	WIDTH cm	HORIZONTAL SURFACE AREA cm^2	INITIAL ANIMAL DENSITY g/cm^2
#/tray				
8	13.5	8.2	110.0	0.011
6	13.5	17.0	229.5	0.019
6	27.0	17.0	459.0	0.010
6	40.5	17.0	688.5	0.005

1/ Water depth in each holding tray remained constant at approximately 7 cm except during periods of tank flushing/cleaning.

2/ Protective substrates were placed in one-half of the compartments in each holding tray.

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TABLE 2. COMPOSITION OF PELLETED DIETS FED DURING EXPERIMENTS I AND II. [1]

INGREDIENTS	EXPERIMENT	
	I	II
	%	%
Menhaden Fish Meal	24.33	32.31
Soy Proteinate	5.82	10.11
Instant Clear Jel (Starch)[2]	29.53	32.26
Alfalfa Leaf Meal	15.00	–
Solka Floc Cellulose	5.00	5.00
Menhaden Oil/Corn Oil Premix[3]	6.02	6.02
Gelatin	4.00	4.00
Sodium Alginate	3.00	3.00
Glucosamine.HCl	3.00	3.00
Mineral Mix	1.50	1.50
Vitamin Premix[5]	1.50	1.50
Choline.Cl Premix[6]	1.00	1.00
Cholesterol	0.30	0.30

[1] On an air dry basis.
[2] Modified food grade starch available from National Starch and Chemical Corp., Bridgewater, N.J. The use of trade names is merely to facilitate descriptions; no endorsement is implied.
[3] Composed of 67% crude menhaden body oil and 33% corn oils; served as a carrier to provide 200 mg dl, α-tocopherol acetate, 200 mg ethoxyquin and 30 mg crystalline β-carotene per kilogram of air dry diet.
[4] Bernhart-Tomarelli mineral mixture (#170750) available from ARS/Sprague Dawley Division, Mogul Corp., Madison, WI.
[5] See Biddle et al. (1) for description of premixes.
[6] Premix consisted of 75% Solka Floc Cellulose and 25% Choline.Cl.

FIGURE 1. SCHEMATIC REPRESENTATION OF ONE OF THREE COMPARTMENTED ANIMAL HOLDING TRAYS USED IN EXPERIMENT I.

WITHOUT SUBSTRATE
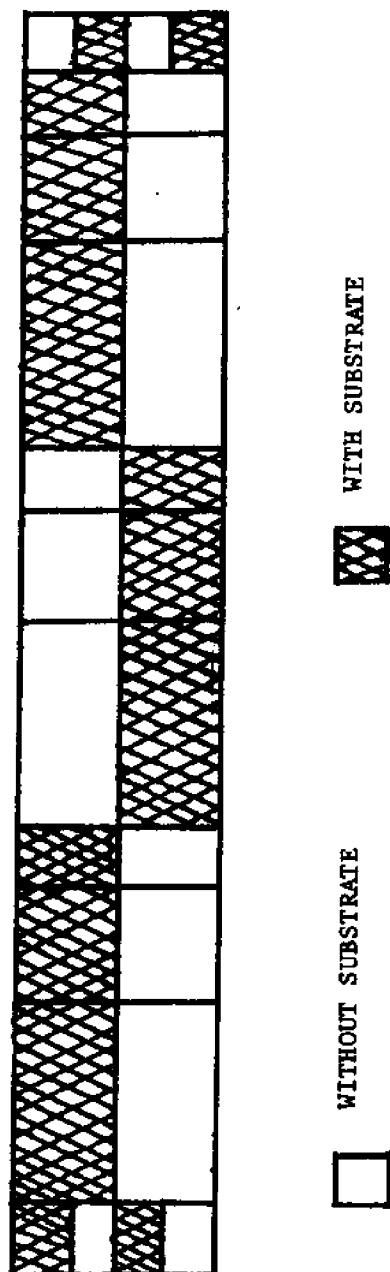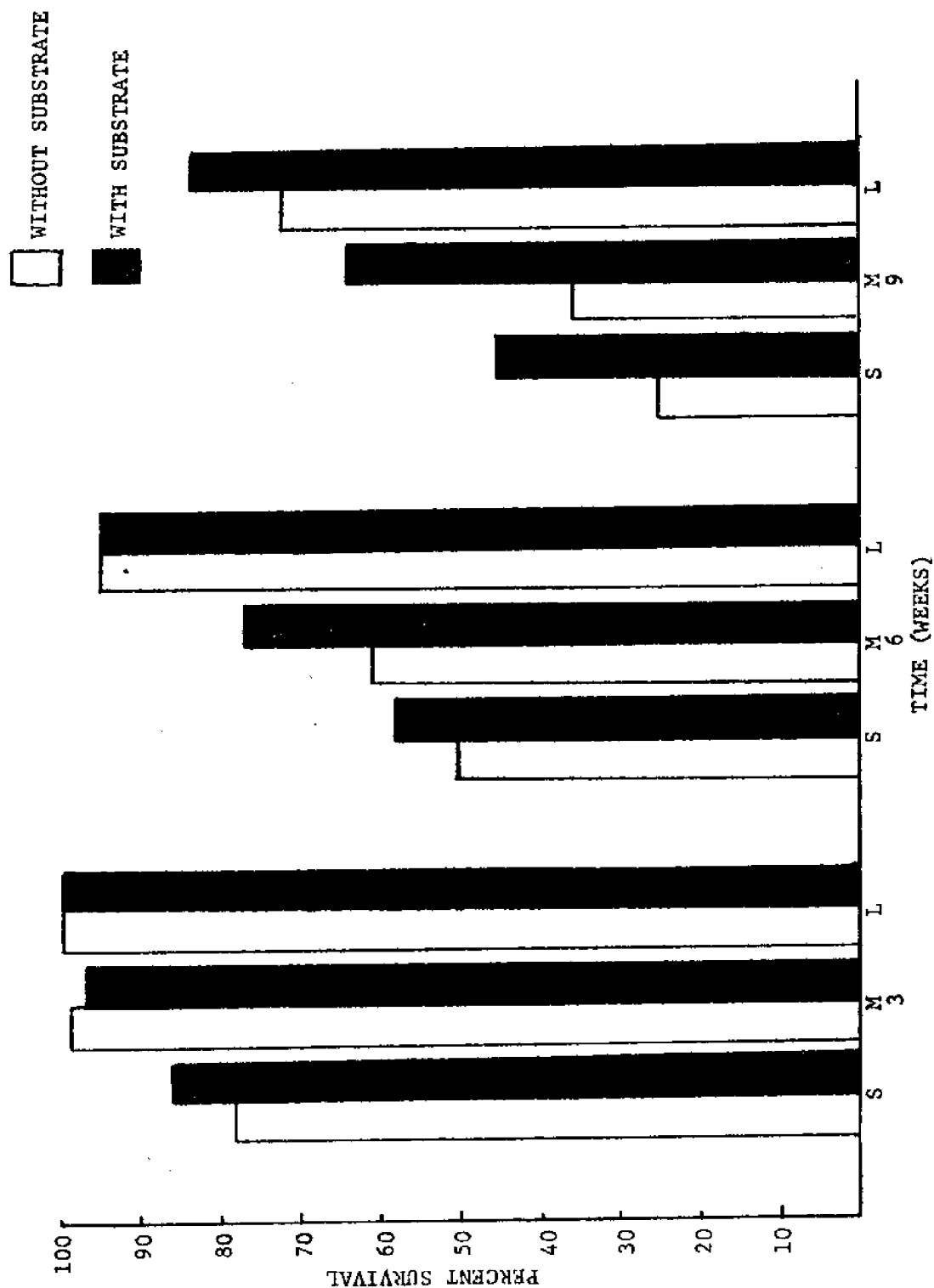
WITH SUBSTRATE

FIGURE 2. EFFECT OF SUBSTRATE AND COMPARTMENT SIZE ON SURVIVAL IN Macrobrachium rosenbergii (Compartment size: S = 230 cm^2; M = 460 cm^2; L = 689 cm^2).

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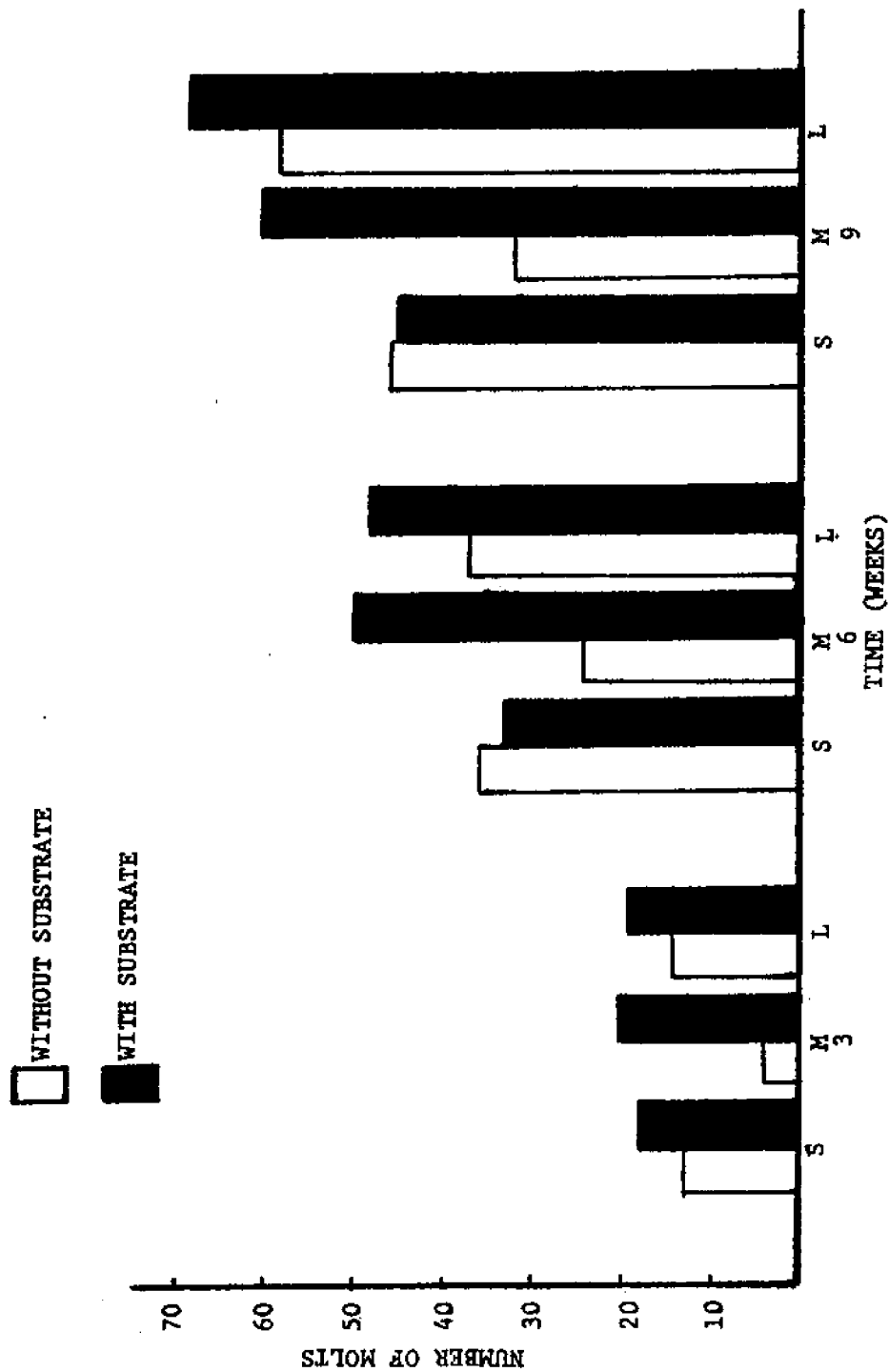
FIGURE.3. EFFECT OF SUBSTRATE AND COMPARTMENT SIZE ON THE MOLTING FREQUENCY OF <u>Macrobrachium</u> <u>rosenbergii</u> (Compartment size: S = 230 cm^2; M = 460 cm^2; L = 689 cm^2).
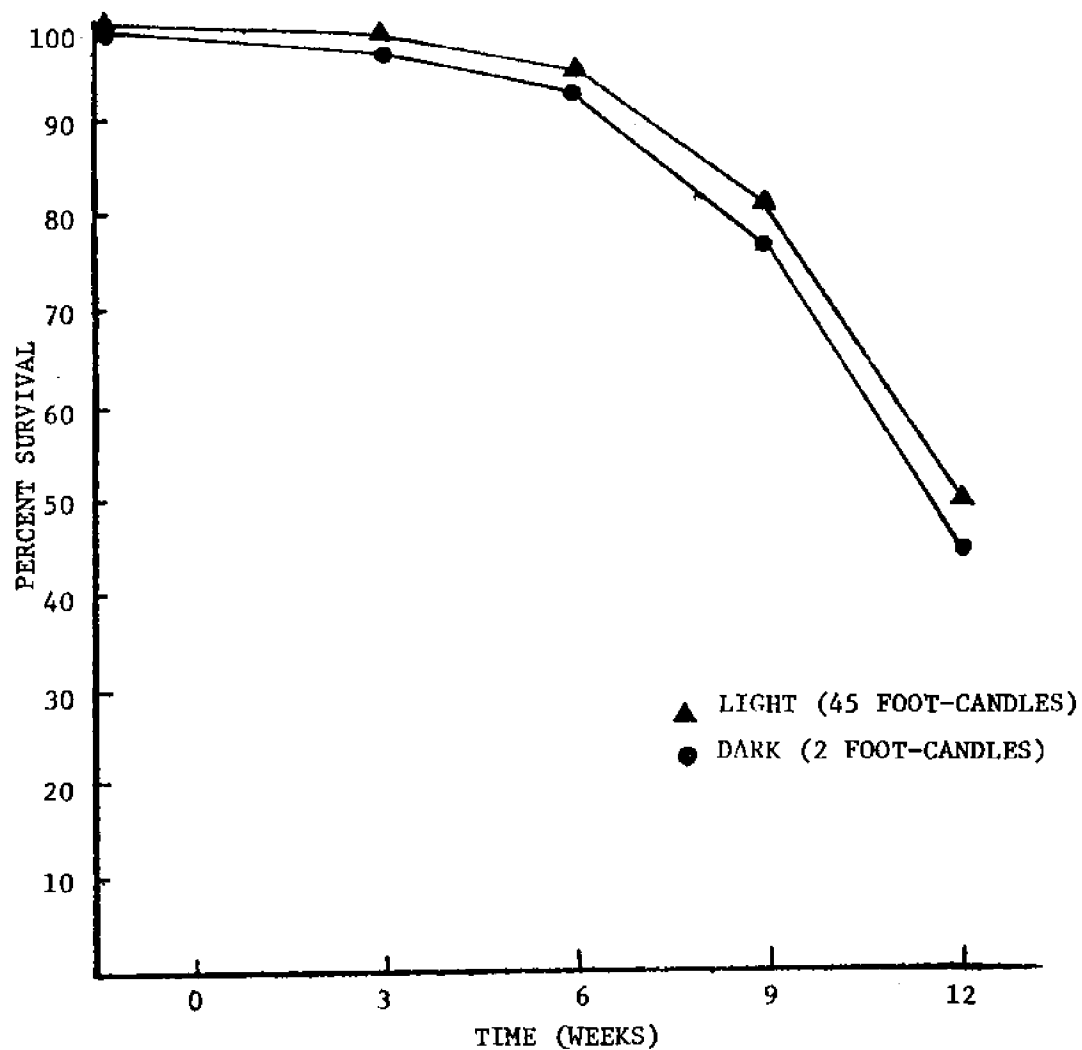
FIGURE 4. EFFECT OF TWO EXTREMES OF LIGHT INTENSITY ON
THE SURVIVAL OF Macrobrachium rosenbergii.
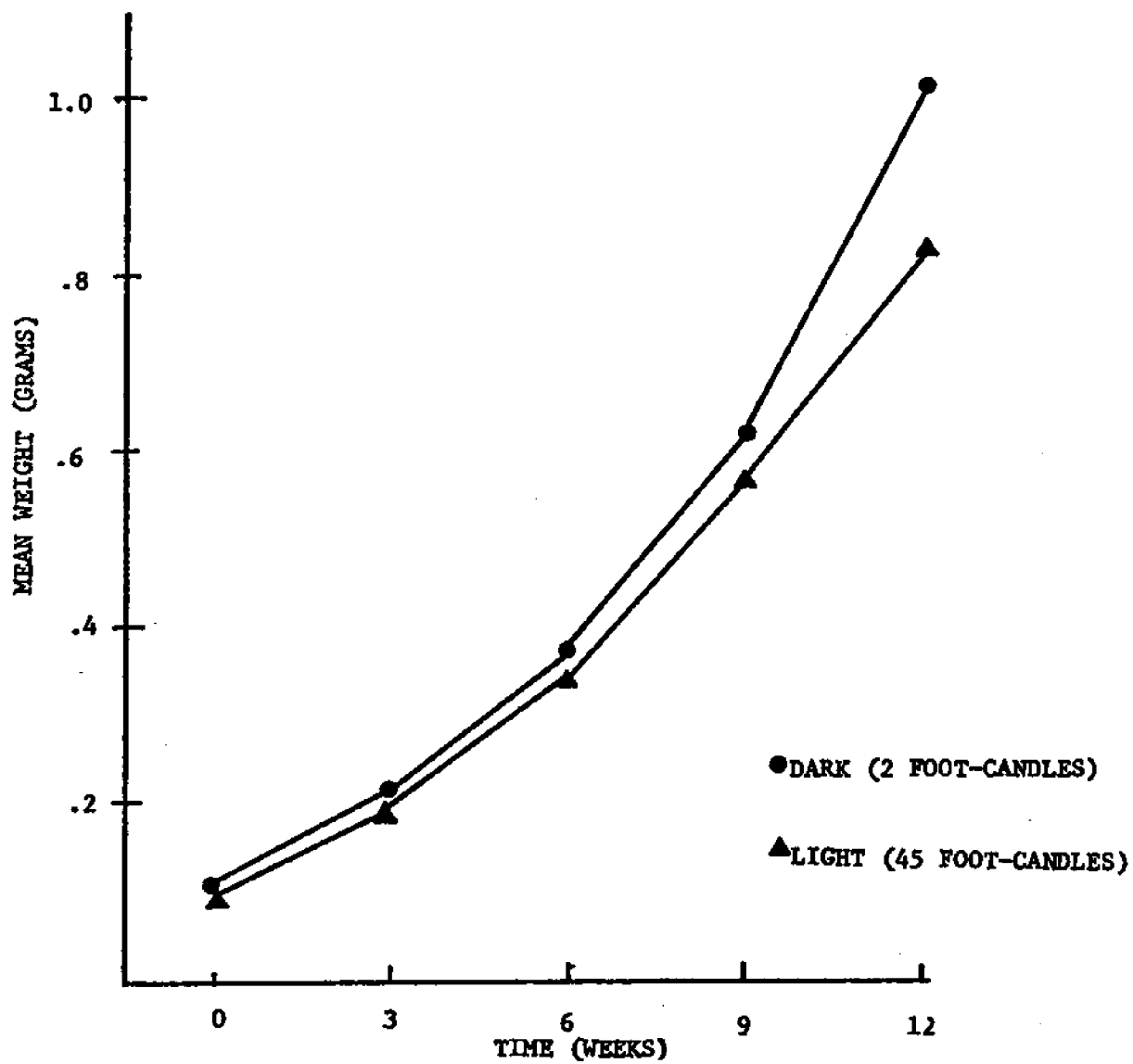
FIGURE 5. EFFECT OF TWO EXTREMES OF LIGHT INTENSITY ON THE
GROWTH OF Macrobrachium rosenbergii OVER A 12-WEEK PERIOD.
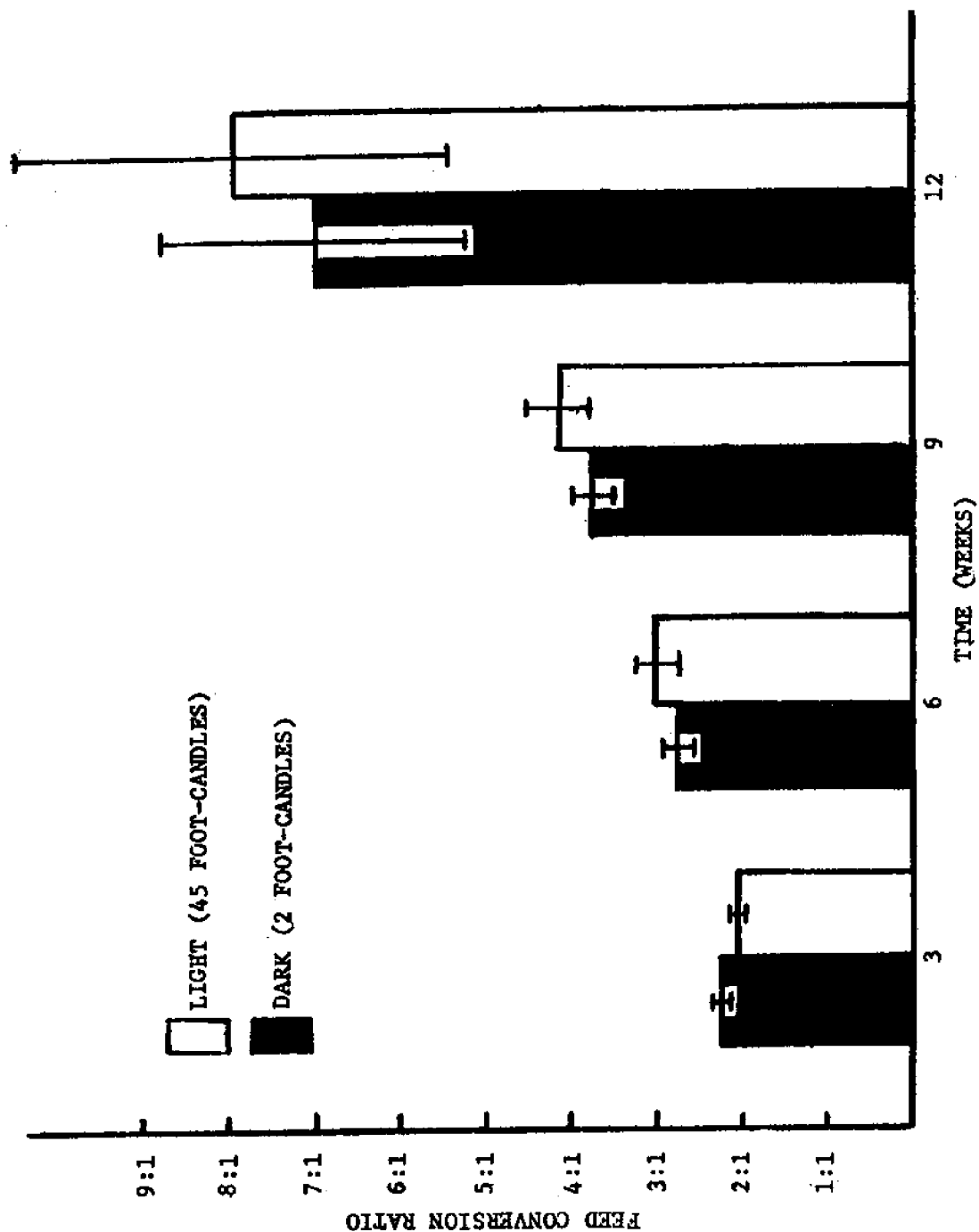
FIGURE 6. EFFECT OF TWO EXTREMES OF LIGHT INTENSITY ON FEED CONVERSION RATES (MEAN VALUES ± STANDARD ERROR OF THE MEANS).

VARIATIONS IN DISSOLVED OXYGEN CONCENTRATION
IN MARICULTURE PONDS - A PRELIMINARY MODEL

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The dissolved oxygen content of shrimp mariculture pond water fluctuates throughout a growing period. Some researchers (Parker et. al., 21) have observed concentrations as low as one part per million, while others (Salser, 31) observed the dissolved oxygen to be more than 250% saturation. At levels below 5 ppm, the growth and survival decrease (Spotte, 34) while at levels above saturation the shrimp is predisposed toward gas bubble disease (Salser, 31).

The factors affecting the concentration of dissolved oxygen in marine systems have been identified and discussed by several authors, but no one has previously consolidated this research so that the importance of each parameter and interactions of these parameters in their normal ranges can be described. The pond manager has had to surmise which of the factors are important in order to decide which he will monitor and control.

The purpose of this paper is to combine the available research on oxygen supply and demand to identify the important "sources" and "sinks" for dissolved oxygen in a mariculture system and to formulate a preliminary model useful to pond managers for pond management.

The factors discussed in this paper are photosynthesis, phytoplankton respiration, shrimp respiration, wind velocity, water exchange rate, and rate of organic decomposition. Photosynthesis, wind and water exchange are the sources while phytoplankton and crustacean respiration with organic decomposition are the evacuation possibilities or sinks.

The objectives of this study were to:

1. Identify the possible "sources" and "sinks" for oxygen in a pond mariculture system.

2. Identify the environmental parameters affecting oxygen concentration in a marine system.

3. Determine the dominant parameters in the system to delineate the variables which have the greatest effect on oxygen concentration.

4. Formulate mathematical equations representing each "source" or "sink" of oxygen and translate these into a preliminary model for predicting oxygen variations.

5. Simulate the oxygen variations over a period of time.

6. Compare the model to pond data and evaluate how well the model compares to actual changes in the environment.

For twenty years scientists have investigated cultured shrimp as a possible commercial food source. Shrimp mariculture has been accepted in Japan where the selling price is very high. These shrimp are usually cultured in the rice fields after the rice growing season is over. The combination of low capital investments and high market prices insure a profit even though the reliability of production is low. In the United States where there is a larger capital investment and a lower market price, commercial shrimp farming has not been found economically feasible. The unreliability of shrimp mariculture is the obstacle to commercial investment.

Experimentation has progressed using two general types of systems: extensive mariculture where only a few of the environmental variables are controlled (Parker et. al., 21) and intensive mariculture where as many variables as possible are controlled (Salser et. al., 31). In intensive mariculture, experiments have proven successful with both open systems where fresh water replaces the systems water (Salser et. al., 31) and closed systems where the water is cleaned and recirculated (Mock et. al., 19). In these systems the dissolved oxygen concentration goes tremendously high because of large volumes of water movement, while in extensive systems, the concentration can go very low because of a large population of phytoplankton. On cloudy days and at night, the phytoplankton respire, but do not release oxygen by photosynthesis (Rawson, 24). This may cause the oxygen level to drop below a critical level for sustaining the shrimp. Spotte (34) stated that the critical level should be considered as one part per million less than the saturation concentration. Therefore, oxygen depletion condition will be defined as an oxygen level more than one part per million less than the oxygen concentration at saturation.

MATERIALS AND METHODS

A preliminary model for the prediction of dissolved oxygen concentration in a shrimp mariculture pond is shown in Figure 1. A major part of this model is the prediction of total oxygen change using the following formula:

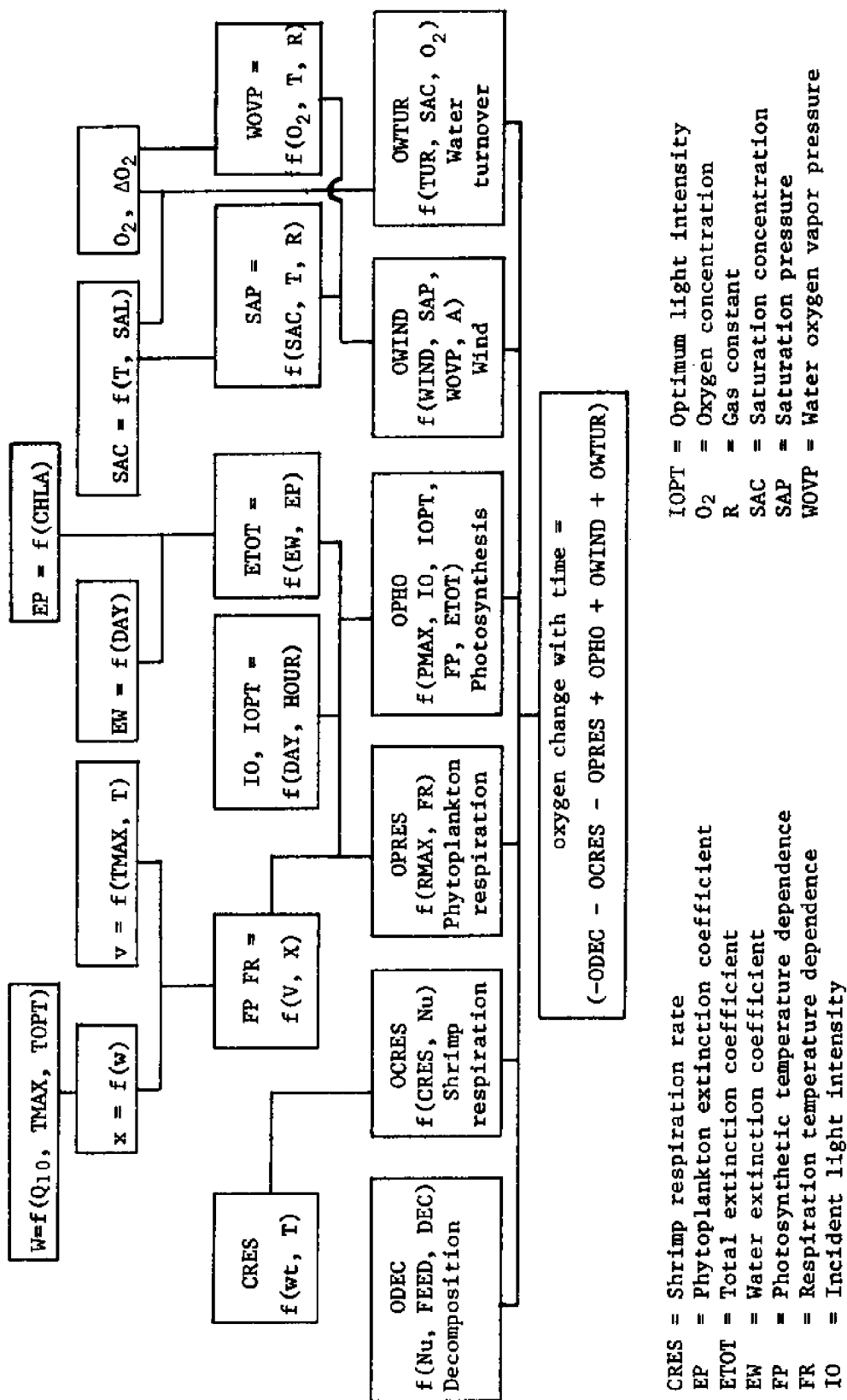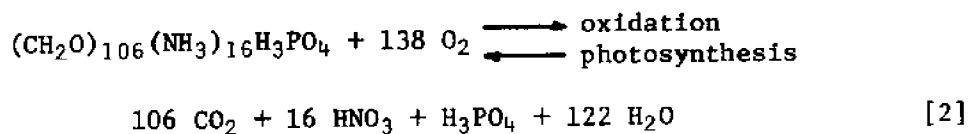$$\frac{\Delta O_2}{\Delta t} = (-ODEC - OCRES - OPRES + OPHO + OWIND + OWTUR) \quad [1]$$

Figure 1. Preliminary model for predicting dissolved oxygen concentration in a shrimp mariculture pond.

Each of the values on the right hand side of the equation represents a rate of oxygen change per unit time (in this study one hour intervals will be used). Each of these rates will be described in detail in the following sections. The $\Delta O_2/\Delta t$ term represents the change in oxygen concentration for the given period.

ODEC is the rate of oxygen used by decomposing organic material on the bottom. This becomes a problem when the shrimp are fed artificial feed. The shrimp will not eat all of the feed presented to them. Experiments show that shrimp presented with thirty percent of their biomass in feed each day will leave ninety percent uneaten (Sick, 32). If less than thirty percent is fed, the proportion left drops until the shrimp can no longer find it or the shrimp are undernourished. As the food on the bottom is broken down, oxygen is used to oxidize the compounds to carbon dioxide, nitrates, and phosphates.

Park (20) and Richards (28) gave a theoretical relationship between the mass of food decomposing and the amount of oxygen required. The paper provides amounts of oxygen required for an amount of food decomposed. The decomposing food supports the benthic bacteria which will also use oxygen.

Redfield (26) showed empirical correlations of total organic matter to oxygen phosphates, nitrates, and carbon dioxide. A general stoichiometric model was developed which accounts for both formation and degradation of organic material. This decomposition follows the relationship:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138\ O_2 \underset{photosynthesis}{\overset{oxidation}{\rightleftharpoons}}$$

$$106\ CO_2 + 16\ HNO_3 + H_3PO_4 + 122\ H_2O \hspace{2cm} [2]$$

This equation provides a mechanism to determine the oxygen given off at a given carbon assimilation rate. For every gram of carbon assimilated 6.94 grams of oxygen are produced while for each gram of organic material decomposing 1.2 grams of oxygen are needed. The oxygen consumption by decomposition would be:

ODEC = (mass decomposing)(1.24 grams O_2/gram of mass decomposed)

The estimation of the food decomposition is based on the amount of food present in the pond. Feeding regimes are usually based on a percentage of total animal biomass. Sick (32) reported that research crustaceans consumed 10% of the feed if fed 30% of total biomass. For simulating the pond the assumption was made that the shrimp were fed 30% of their biomass, and that 30% of that food was uneaten. The value of the percent decomposing was varied until a realistic value of oxygen concentration was obtained. If one ten-millionth of the uneaten food added each day decomposes, the value of oxygen consumed is equivalent to the amount consumed by phytoplankton.

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OCRES is the rate of oxygen consumption by the shrimp in the mari-culture pond. The shrimp or fish consume oxygen depending on their population density, weight, movement, and the water temperature. Van Den Avlye (39) developed the following model from research done by Beamish (6).

$$OCRES = a_1 \ e^{a_2 s} \ WT^{-a_3} \ e^{a_4 T} \qquad [3]$$

where OCRES is the respiration rate for the fish, s is the swimming speed, WT is the weight of the fish, and T is the water temperature.

The respiration rate of shrimp has been studied by Alcaraz (1) and Subrahmanyam (38). Both of these researchers measured oxygen consumption as a function of body weight of the shrimp. Alcaraz used palaemon species and derived equations relating oxygen requirement to weight for each species and temperature.

$$\log OCRES = a_1 + a_2 \log WT \qquad [4]$$

which transforms into

$$OCRES = a_3 e^{-a_4/T} WT^{a_2} \qquad [5]$$

when combined with the Arrhenius equation. Respiration increases exponentially with temperature and also increases with increasing weight. Subrahmanyam used activity of the shrimp and the weight of the shrimp to predict the oxygen consumption. Two types of Penaeid were used and activity of these were related to the tidal activity. An exponential type function will fit his data:

$$OCRES = a_1 \ e^{a_2 WT} \ e^{a_3 A} \qquad [7]$$

where a_1, a_2, and a_3 are constants for each type shrimp at each temp-erature. On inspection, the activity is insignificant so can be dropped from the expression. Temperature would be significant but was not studied. The equation of Alcaraz was considered accurate enough for use in this study. The effect of the crustacean respiration on the total oxygen change is so small that any inaccuracy in this equation would be insignificant for this model.

OPRES is the rate of oxygen consumption by the phytoplankton. The respiration of phytoplankton has been studied by Lehman (17), Riley (29), and Van Den Avyle (39). Each of these authors calculated the respiration rate as a proportion of the maximum respiration rate. Lehman (17) used a modification of an equation presented by Riley in which the respiration was dependent on the water temperature and the amount of carbon stored within the cell. This function accounts for the increase in respiration as the cells become more mature and store more carbon. Often the amount of carbon stored at any time is not known so another approach is necessary. Van Den Avyle (39) estimated

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respiration as a proportion of the maximum respiration dependent on the temperature.

Park (20) formulated an empirical function to predict the temperature effect on respiration and photosynthesis. This is as follows:

$$F(t) = V^x e^{x(1-V)} \qquad [7]$$

$$V = (TMAX - T)/(TMAX - TOPT) \qquad [8]$$

$$x = \frac{W^2 (1 + \sqrt{1 + 40W})^2}{400} \qquad [9]$$

$$W = \ln sq (TMAX - TOPT) \qquad [10]$$

For these equations, TMAX is the upper lethal limit, TOPT is the temperature at which maximum respiration occurs, T is the temperature of the water and sq is the Q_{10} value.

F(t) is in the interval (0,1.0) and F(t) = 1.0 when T = TOPT. This is a multiplicative factor to decrease the phytoplankton respiration from its maximum which occurs at TOPT.

If the temperature of the water exceeds the optimum temperature, the temperature dependence is assumed to be unity. This gives a maximum respiration rate for any temperature above the optimum.

The respiration of the phytoplankton is represented by a function used by Van Den Avyle (39):

$$OPRES = RMAX \times FR \times 6.9434 \ mgO_2/mgC \qquad [11]$$

where MRAX is the maximum possible respiration rate. Lehman (17) lists the maximum rate of respiration and photosynthesis for four types of phytoplankton. The maximum respiration rate is approximately equal to .2(PMAX). This gives a value of .3 mgC/mgPHY-hr. The amount of phytoplankton present in the pond was assumed to be .02936 mgCHLA/liter. The oxygen in the pond is very sensitive to the amount of phytoplankton, To convert from mg chlorophyll a to mg of phytoplankton (Bannister, 5) suggested a value of .0114gPHC/mgCHLA.

Each species of phytoplankton has a different maximum respiration rate and temperature dependence. The Q_{10} value would vary for each type of phytoplankton. The phytoplankton in the system is not all the same species, nor is one species dominant throughout the growing season. The wide variety of phytoplankton species keep the total characteristics of the phytoplankton in the pond rather constant. The phytoplankton in the pond, therefore, is assumed to be a homogenous mass containing the average characteristics of the phytoplankton present. The Q_{10} value was chosen to have a value of two (2).

OPHO is the rate of oxygen accumulation due to photosynthesis. Photosynthesis is a major source of dissolved oxygen to waters and has been studied extensively in the past with a recent review by Van Den Avyle (39). In this paper, equations were formulated to model photosynthesis as a function of light intensity and temperature and included a feed-back inhibition when the intensity of light is too great. An optimum intensity of light produces a maximum rate of photosynthesis while intensities above or below this optimum cause a decrease. There are numerous production equations for photosynthesis. Vollenweider (41) reviewed the literature prior to 1965. More recent equations have overcome some of the inaccuracy of earlier work. Steele (35), Fee (12), Bannister (5), and Lehman et. al. (17) have each presented equations for photosynthesis. The most used equation is the equation presented by Steele (35) because of its simplicity and accuracy. If this equation is integrated over the depth of the pond at a point, according to Beers Law – $IZ = IO[e^{-ETOT(Z)}]$, the following equation results:

$$PHO = \frac{PMAX(e)}{ETOT} e[(\frac{IO}{IOPT} e^{-ETOT(Z)} -(\frac{IO}{IOPT})] \qquad [12]$$

This equation has the same units as PMAX and gives the photosynthesis rate for a volume of water. The depth of the column is Z and the light attenuation is defined as the extinction coefficient [ETOT]. The optimum intensity is peculiar to the type of phytoplankton present while the intensity of light falling on the pond is dependent on the time of day, time of year, and the cloud cover.

Vollenweider (41) assumed that the light intensity varied throughout the day according to

$$IO = 0.5 \ IOMAX \ (1 + Cos \ 2\pi t') \qquad [13]$$

The t' in this equation is a characteristic time of day representing the hours from sunrise divided by the total hours of the day. IOMAX is the maximum intensity for the day at noon. This value varied throughout the year as defined by Lehman et. al. (17) as:

$$IOMAX = IW + IS \ [1 - Cos \ (\pi(Day + 8)/180)] \qquad [14]$$

This function predicts a minimum intensity at winter solstice and a maximum intensity at the summer solstice. Since the units for this equation are Langleys, the final intensity will be in Langleys also.

As the depth Z increases the intensity of light decreases. The value of ETOT is dependent on the amounts of phytoplankton and particulate matter in the water. Van Den Avyle (39) expresses this as:

$$ETOT = EPHY + EWAT \qquad [15]$$

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The extinction coefficient for the phytoplankton was described by Bannister (5) as

$$EPHY = KcCHLA \qquad\qquad [16]$$

where Kc is a proportionality constant and CHLA represents the concentration of chlorophyll a. The extinction coefficient for the water is dependent on the water turbidity, and is time varying.

The final photosynthetic rate OPHO is

$$OPHO = PHO \times FP \times 6.9434 \ mgO/mgC$$

where FP is the temperature factor for photosynthesis calculated by Park's relationship given in equation [7].

Parameters for phytoplankton were estimated from data that has been used in research with marine systems. Calculations for the carbon uptake (equation 12) required estimation of the maximum rate of carbon uptake and the light intensity at which the maximum rate occurs. The gross carbon uptake for different types of phytoplankton are given by Lehman et. al. (17). The values vary for each type of phytoplankton. Blue green algae has a value of 1.5×10^7 μ moles O_2/cell hr. while dinoflagellates have a maximum of 15×10^7 μ moles O_2/cell hr.

Van Den Avyle (39) used a value for the combination of different types of phytoplankton. PMAX was estimated at 0.15 g. carbon assimilated/ hr. mg. phytocarbon. This value would change throughout the year as the phytoplankton adapted to changes in temperature or light intensity. The optimum intensity for PMAX was assumed to vary throughout the season and was assumed equal to 0.25 (IOMAX) with a thirty day lag.

OWIND is the rate of oxygen transfer between the air and water. The driving force for diffusion between the water and atmosphere is the partial pressure difference between the two. The amount of oxygen that the water can hold is referred to as the solubility; the solubility of a gas in salt water decreases with increasing temperature and salinity of the water. Kester (16) presented the following empirical equation for determining the solubility concentration of oxygen in marine water at any salinity and temperature while the atmospheric pressure is one atmosphere with a 100% relative humidity:

$$lnSAC = a_1 + a_2 \ (100/T) + a_3 ln(T/100) + a_4 (T/100) +$$

$$SAL \ [a_5 + a_6(T/100) + a_7(T/100)^2] \qquad [18]$$

The oxygen added by the wind is discussed in detail by Kester (16). A mass transfer equation relating change in oxygen concentration to the partial pressures of oxygen in the water and in the air and an exchange velocity is

$$\frac{d \ O_2}{dt} = \frac{A \ EO_2 \ (P_{air} - P_{water})}{KO_2} \qquad [19]$$

where A is the area of the pond exposed to the atmosphere. EO_2 is the exchange velocity for oxygen and is dependent on the boundary layer or wind velocity. KO_2 is the Henry's constant for oxygen in that water.

The exchange velocities have also been investigated by Downing and Truesdale (11), Redfield (25), Pytokowicz (23) and more recently by Broecker and Peng (8). With these studies a value for the exchange velocity has been established. Kester presented a graph for the exchange velocity versus wind velocity. The function can be represented by the equation:

$$EO_2 = 1.235 \ e^{.115(wind)} \ x \ 1000 \qquad [20]$$

The wind will be augmenting the level of oxygen in the pond in most cases. The direction of oxygen transfer can reverse if the partial pressure of oxygen in the water becomes greater than its partial pressure in the air.

Another method of aeration is to bubble air or oxygen through the water (21, 19). As the air bubble rises to the top, oxygen diffuses from the bubble into the water. Other gases which have higher vapor pressure in the water than in the air diffuse from the water into the bubble. This can be represented as a mass balance equation similar to that used by Kester for wind with the notable difference being that the exchange velocity would be much greater due to smaller boundary layers in rising bubbles (Bird et. al., 7) and (Kester, 16).

OWTUR is the net amount of oxygen carried into or out of the pond with the circulating water per unit time.

There are several procedures that pond managers take to prevent the occurence of oxygen depletion. The easiest method is to let fresh saturated water into the pond, forcing the oxygen depleted water out the drain. Once the water enters the pond it mixes with the pond water thereby raising the oxygen content of all the water. The water flushed out the drain is at the same concentration as the pond water.

The oxygen entering the system with the inlet water is dependent on the rate of water turnover and the concentration of oxygen in the pond water. The inlet water is saturated due to the oceanic source and aeration during the pumping process.

$$OWTUR = TUR \ (CIN - COUT) \qquad [21]$$

Since the equations are for a unit volume of water, the turnover rate must be expressed as a percent of the water changed per unit time. CIN is the concentration of oxygen in the inlet water and COUT is the oxygen concentration in the drained water.

RESULTS AND DISCUSSION

Sensitivity analysis

In order to carry out an analysis of the influence of each parameter on the total change of O_2 during a three day period, the following key values were chosen as they simulated a pond where O_2 remained almost constant throughout the period: number of shrimp per liter, NU, 0.012 (this is equivalent to the shrimp population density reported by Salser, 31); weight of shrimp, WT, 20.0 grams (Salser, 31); feed rate (fraction of biomass per day), FEED, 0.3 (Sick, 32); wind velocity 10 meters above the surface, WIND, 1 m/sec (Kester, 16); temperature, T, and salinity, SAL, 293°K and 30 ppt respectively; percent illumination due to cloudiness as compared to a maximum daylight situation, CLO, 0.5; amount of chlorophyll a, CHLA, 21936 x 10^{-2} mg/liter (unpublished data from shrimp mariculture pond); percent of food not eaten decomposing (DEC), 1 x 10^{-8}; water turnover rate (TURN), 0.02 percent per hour.

The pond is assumed to have an area of 4.44 x $10^4 m^2$ and a depth of 0.75 m; the particular days chosen are the 153rd, 154th, and 155th of the year. The initial O_2 value is the saturation level at T = 293°K and SAL = 30 ppt. Several parameters were varied one at a time to determine their individual effect while holding all other parameters constant.

Figure 2 contains oxygen concentration curves from two actual shrimp ponds as well as modeled oxygen concentrations at a high and a low level of chlorophyll a, CHLA. Note that high CHLA produced much greater variations in oxygen due to higher photosynthesis and higher respiration rates. Initial oxygen concentrations for the "HI CHLA" and "LOW CHLA" curves were arbitrarily established, the "HI CHLA" appeared to more accurately represent actual data.

Figure 3 shows the sensitivity of the total model to a change in the mass decomposing one hour. Notice that changing the rate of decomposition by a factor of ten will either saturate or deplete the oxygen content in the pond. (This graph shows the simulation profiles for three separate decomposition rates.) Even while the pond is going to saturation or depletion, the cyclic effect caused by photosynthesis is still evident.

Figure 4 presents the effect of light transmitted through clouds on the change in oxygen concentration at the end of a three day period. This shows the sensitivity of the model to a change in light intensity. One hundred percent light transmitted is the case of no clouds. Photoinhibition was not seen because the rate of photosynthesis was calculated for the pond bottom where light intensity was below the optimum for photosynthesis. At the top surface of the pond the maximum increase in ΔO_2 would occur at 50% light transmittance which was assumed to be the optimum light intensity for photosynthesis. For the overall model, the effect of depth below surface was taken into account by integrating over the pond depth.
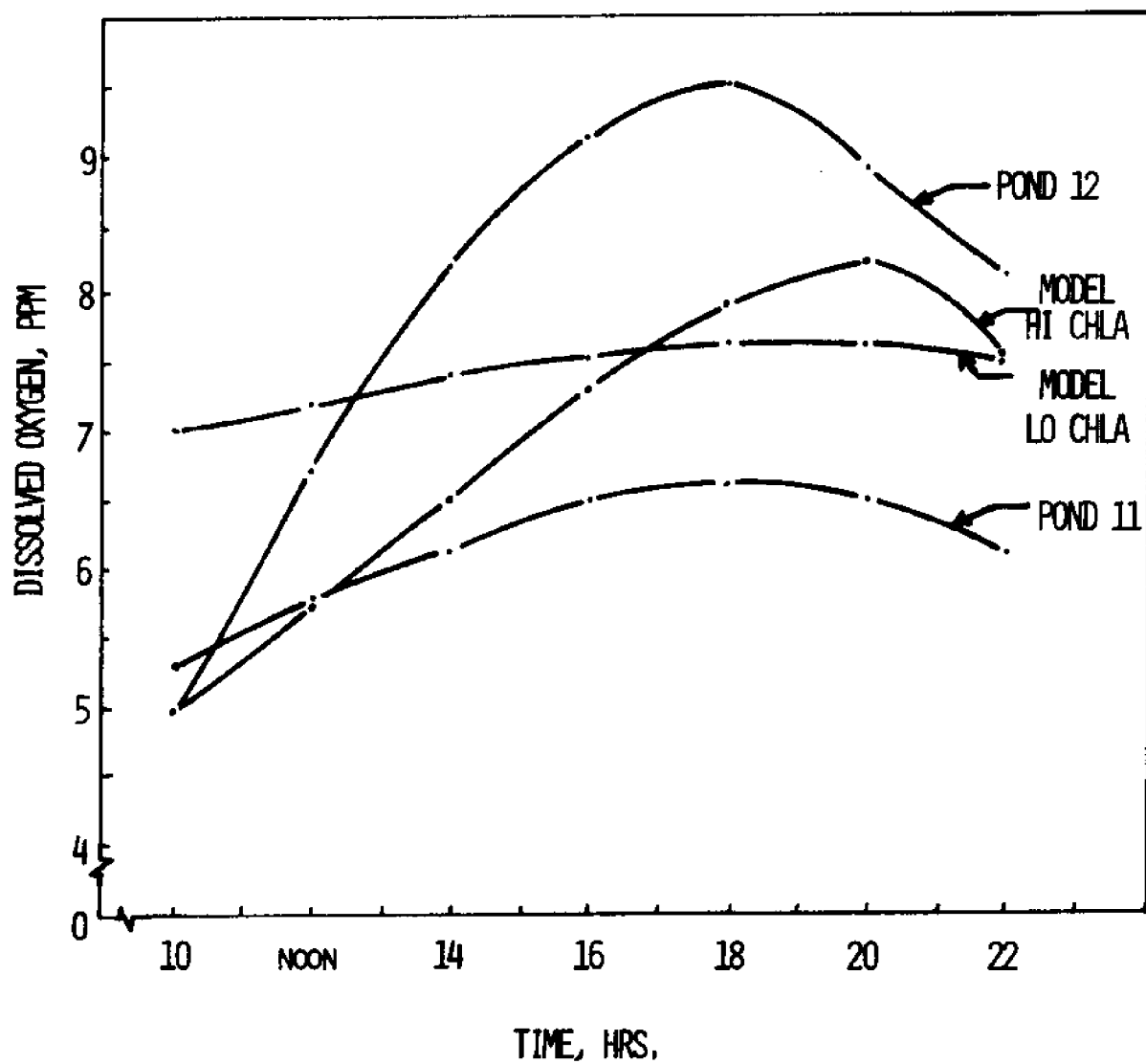
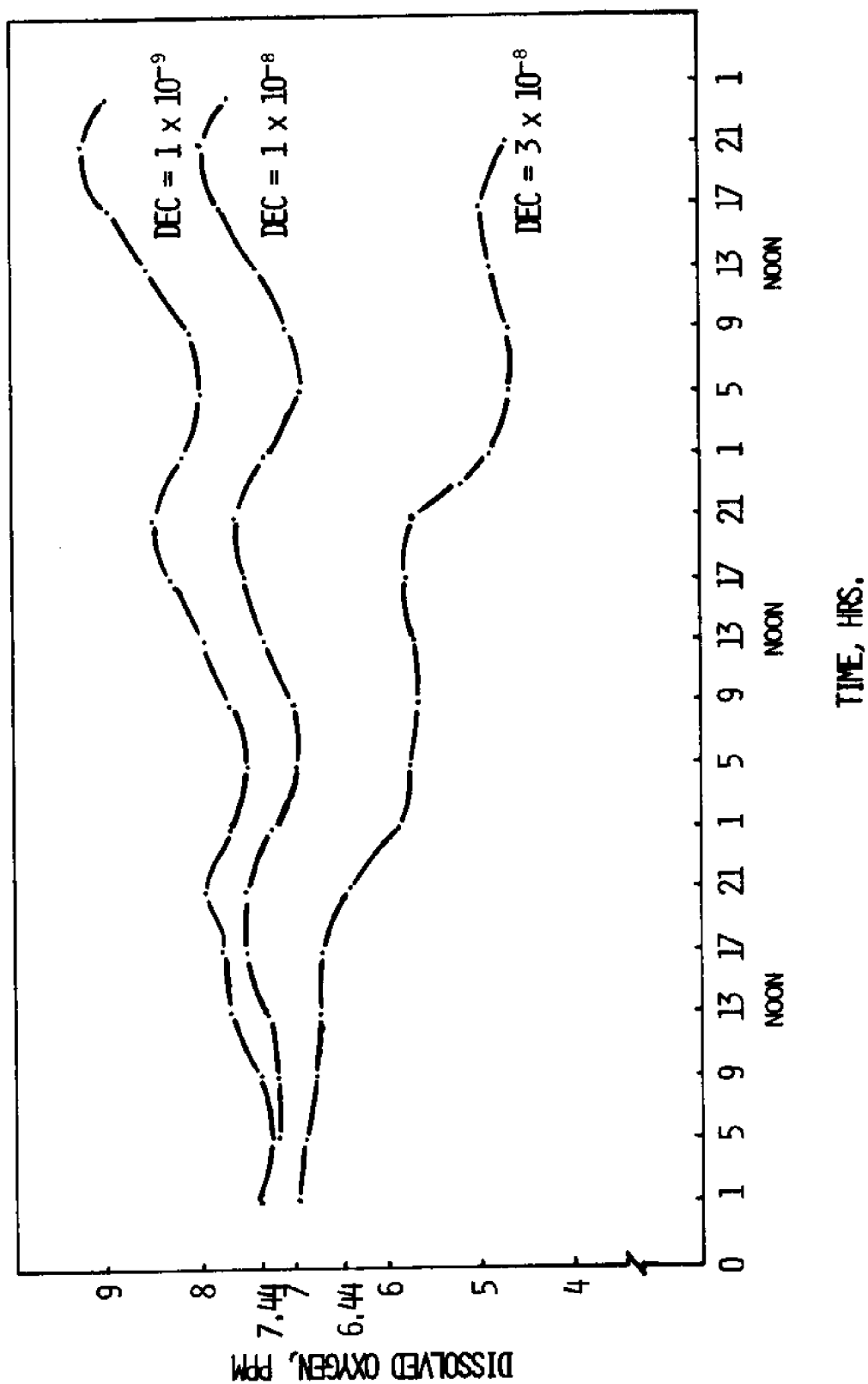Figure 2.   Oxygen profile in shrimp ponds.

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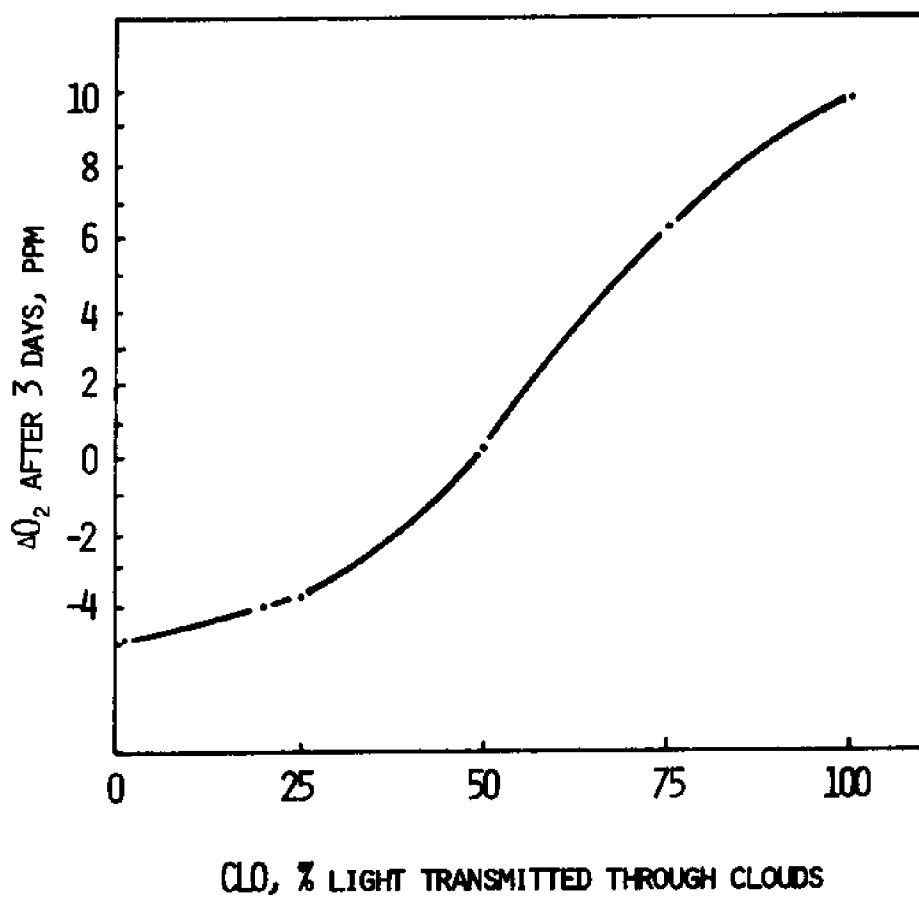Figure 3. Effect of feed decomposition rate on oxygen level.

Figure 4.   Effect of light transmittance on change in oxygen concentration.

FEED, tested for a wider range of values than those representing actual situations, gave an overall O_2 change of approximately 5 ppm (see Figure 5). Although this ΔO_2 value would vary considerably with a change in DEC, for this particular case it is secondary in importance to both CLO and DEC.

The effect of changing shrimp population density, NU, was minimal even though relatively large shrimp were being simulated. This is shown in Figure 6. The oxygen requirement for respiration was negligible compared to phytoplankton respiration.

Even less important was WIND. An overall ΔO_2 of 2×10^{-16} ppm was obtained when WIND was varied using zero values for every other parameter except water conditions and 10 m/sec for wind velocity. The dominant effect of the wind may be in the prevention of stratification of the pond water into an oxygenated surface and an oxygen-depleted sublayer. The friction of the wind causes the top layer of water to be turned to the bottom and thereby bringing oxygen deficient water to the top.

Needs for further research

The information in this paper represents a preliminary model. The parameter estimation was based on numerous sources. To correctly represent a mariculture system, the parameters must be obtained experimentally from that system. Research into the number and types of benthic bacteria would help to delineate the decomposition rate. The numbers and types of phytoplankton in the pond are needed to determine the photosynthesis rate as well as the phytoplankton respiration rate. The climatology of the area in which the ponds are located will affect the light intensity, cloud cover, and the temperature of the water. This information needs to be detailed and included in the model. The effects of temperature, salinity, and shrimp weight on the respiration rate of the types of shrimp cultured need to be found. This should be performed in factorial type experiments so the interactions can be identified.

CONCLUSIONS

1. The major sources of oxygen in a shrimp pond are photosynthesis and water exchange.

2. The major oxygen sinks in a shrimp pond are phytoplankton respiration and decomposition of organic material. Shrimp respiration required a negligible amount of oxygen.

3. The major factors which affect oxygen concentration in a shrimp pond are decomposition of organic material, amount of chlorophyll a present, amount of sunlight, water exchange rate, water temperature and salinity, and the feeding rate.

4. It is possible to simulate oxygen concentration in a shrimp pond; however, more research is needed before such a simulation can be done accurately.

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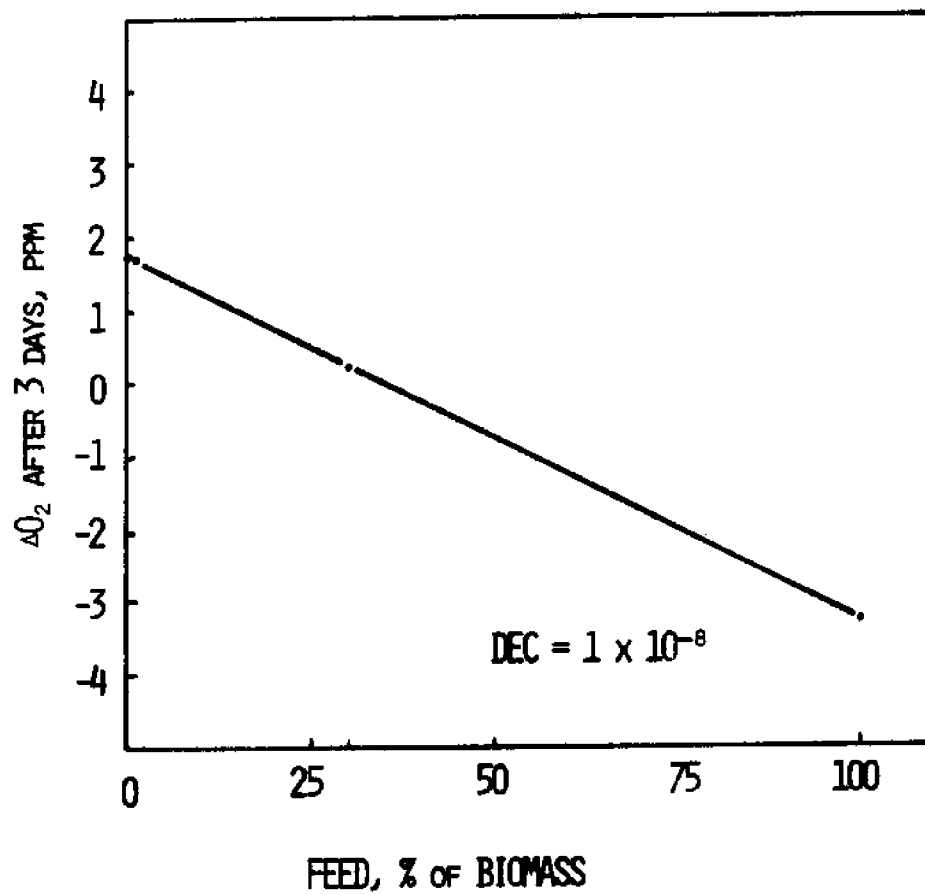
Figure 5. Effect of feeding level on change in oxygen concentration.
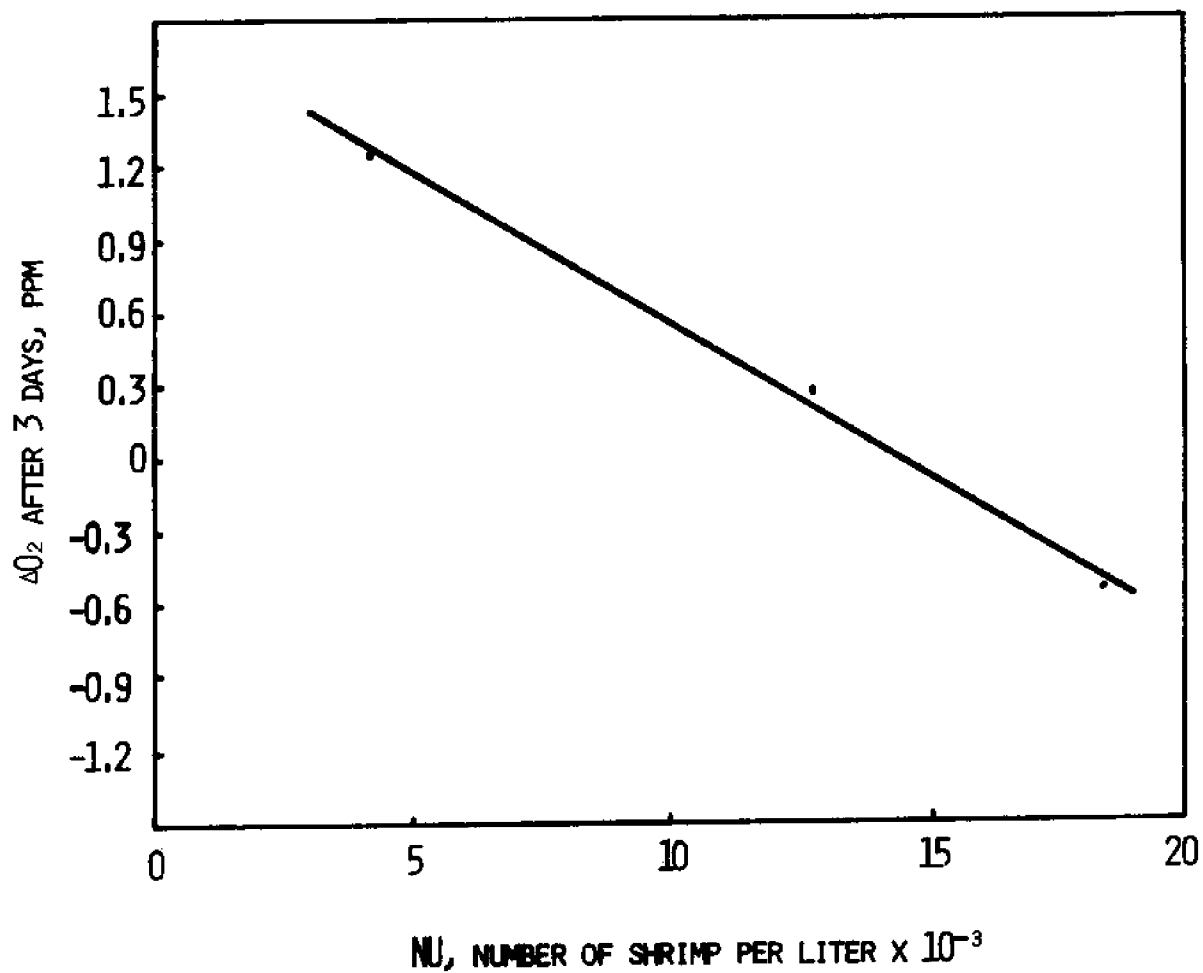
Figure 6. Effect of shrimp population density on change in oxygen concentration.

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APPENDIX A. List of Symbols

A   activity or area
$a_{1,n}$ constants
CHLA   chlorophyll $\underline{a}$
CIN   oxygen concentration of water entering pond
COUT   oxygen concentration of water exiting pond
EO_2 exchange velocity for oxygen
EPHY   phytoplanktonic extinction coefficient
ETOT   total extinction coefficient
EWAT   nonphytoplanktonic extinction coefficient
$F(t)$ temperature dependence
FP   temperature dependence of photosynthesis
FR   temperature dependence of respiration
IO   incident light intensity
IOMAX   maximum light intensity for one day
IOPT   optimum light intensity
IS   intensity at summer solstice
IW   intensity at winter solstice
IZ   intensity at depth
Kc   proportionality constant
KO_2 Henry's constant
O_2 oxygen concentration
OCRES   rate of oxygen for shrimp respiration
ODEC   rate of oxygen for decomposition
OPHO   rate of oxygen from photosynthesis
OPRES   rate of oxygen for phytoplankton respiration
OWIND   rate of oxygen from diffusion
OTUR   rate of oxygen from water turnover
P_{air} partial pressure of oxygen in air
P_{water} partial pressure of oxygen in water
PHC   photoplanktonic carbon
PHO   carbon assimilation rate
PMAX   maximum photosynthetic rate
ppm   parts per million
RMAX   maximum respiration rate
s   swimming speed
SAC   saturation concentration
SAL   salinity
SAP   saturation pressure
sq Q_{10} value
T   temperature
t' characteristic time of day
TMAX   maximum sublethal temperature
TOPT   optimum temperature
TUR   percent water turnover per hour
Vol   volume of pond
WT   weight of shrimp
Z   depth below surface

AN EVALUATION OF POWER PLANT EFFECTS ON INITIAL PATTERNS OF FISH DISTRIBUTION IN A SMALL FLORIDA ESTUARY

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INTRODUCTION

There is a great deal of concern over the potential detrimental effects on fishes attracted to or repelled from thermal discharges from power plants. Although species composition and thermal responses of fishes congregated in various discharge canals have been observed, little is known of the distribution of fishes in and around the thermal plumes. In order to document the effects, if any, which operation of the Anclote Power Plant might have on the aquatic community and ecosystem of the Anclote Anchorage and the Anclote River, Florida Power Corporation (FPC) contracted with several major research organizations to conduct comprehensive biological studies in the vicinity of the intake and in the areas of the Anchorage affected by the discharge. The studies covered the calendar year 1976.

The survey for fish was conducted by Texas Instruments Incorporated. The study program conformed to the format outlined in the Environmental Protection Agency (EPA), Region IV, document for design of 316 demonstrations and responded to stipulations set forth by EPA in a draft permit for the Anclote Plant, Unit 1 (NPDES No. FL0002992).

Observed monthly and spatial trends of fish populations at stations removed from potential plant effects were compared with data from areas adjacent to plant intake and discharge. Comparisons were made between current data trends and preoperational information (6, 1, 2, 3, 7) collected in the vicinity of the Anclote site. Finally, station comparisons of seasonal variations in species number, composition, and abundance aided in determining to what extent juvenile fish utilize the Anclote River as a route to upstream nursery grounds.

Because the time frame necessary for local ecosystems to adjust to the power plant's effects is presently unknown, evaluations were limited. Estuarine fishes are highly mobile and are able to relocate rapidly and utilize available habitats. Alteration of the environment by changes in circulation patterns, currents, temperature gradients and the like involve rapid changes in temporal and spatial patterns of local ichthyofauna. Results reported in this

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study provide the first 1-yr evaluation of power plant effects and
indicate initial patterns of ecological change during this period of
environmental instability.

MATERIALS AND METHODS

Description of the study area

The Anclote Anchorage, in southwestern Pasco County, Florida,
is considered a very productive estuarine system. It is a shallow
coastal lagoon of about 23.3 km^2, and is separated from the Gulf of
Mexico by a series of barrier islands called the Anclote Keys.
Tides are the predominant hydrodynamic force of the Anchorage with
winds playing a secondary role. Currents are primarily tide and
wind driven, the latter being dominant when winds exceed 16.1 km/hr
(15). Temperature and salinity distributions are generally affected
by tidal currents with solar radiation modifying temperature distri-
butions, especially in the shallows.

The Anclote River is the major stream discharging into the An-
chorage. Average flow of the river at its mouth is approximately
38.2 m^3/s but flow is characterized by long periods of low flow (2.8
m^3/s or less) and short periods of high flow, dependent on seasonal
variations in rainfall. The river has a base runoff of approxi-
mately 10% from aquifers.

Seagrasses are the predominant benthic plants in the estuary.
A major portion of the bottom is covered by one or more of several
species: Thalassia testudinium, Syringodium filiforme, Ruppia
maritima, Halodule wrightii, and Halophila spp. The combination of
shallow waters (with depths generally less than 3 m), rich and wide-
spread grass beds, input of freshwater from the Anclote River, and
flushing action of the tides make the Anchorage a very productive
nursery area for sport and commercially important species.

The Anclote Power Plant is located on a 164-ha site just north
of the mouth of the Anclote River and adjacent to the Anclote Anchor-
age. The generating facility currently consists of one oil-fired
steam-electric generating unit with a rated net capacity of approxi-
mately 515,000 KWe. Operating at design capacity, cooling water cir-
culated through the condensers is mixed with dilution water result-
ing in a combined flow of 62.8 m^3/s and a ΔT of 2.8°C at the point
of discharge. Both cooling water and dilution flow are withdrawn
from the Anclote River at a point near the river mouth. The heated
water is discharged to the Anchorage through a dredged channel ex-
tending offshore from the bulkhead line to the 1.8-m depth contour.

Sampling gear and stations

A 75-ft x 6-ft (22.8-m x 1.8-m) nylon-bag seine with 1/8-in.
(3-mm) delta mesh was used to monitor juvenile and small fishes at
five stations in the vicinity of the Anclote discharge and intake
canals and Anclote River (Figure 1). Replicate collections were
taken monthly at beaches near the intake (Station FS02) and the ther-
mal discharge (Station FS03) with other shore stations sampled at
least bimonthly.

To maintain maximum comparability with previously collected
baseline data, materials and techniques used to monitor ichthyofauna

of seagrass beds and the discharge canal conformed to those de-
scribed by Baird et al. (1). The bimonthly program consisted of
day-night replicate sampling with both trammel net and trawl at
three permanent stations (Figure 1). At each seagrass station, a
300-ft x 4-ft (91.4-m x 1.2-m) trammel net with 2-in. (5-cm) stretch
inner mesh and 12-in. (30.5-cm) stretch outer mesh walls, was fished
for 45 min. During this time, a 10-ft (3.1-m) commercial try trawl,
with 1 3/8-in. (3.5-cm) stretch mesh, was towed parallel to the tram-
mel net on each side at a distance of approximately 76.2 m from the
trammel net. Comparable sampling was conducted at a station located
near the midpoint of the discharge canal. An 8-ft (2.4-m) deep tram-
mel net was used to adequately sample the discharge canal. Because
of spatial constraints, modified trawl sampling was conducted in
front of the trammel net.

Data collection and analytical procedures

The total number and weight of each fish species was recorded
for each sample. Standard length and weight were determined for all
individuals, or a random subsample of at least 25 specimens per
species. Lengths were recorded to the nearest 1.0 mm, and weights
to the nearest 0.1 g.

Physical data expected to influence fish catches were rou-
tinely recorded in situ. A Weston and Stack oxygen analyzer system,
with temperature compensation and readout, was used in coordination
with a YSI Model 33 induction salinometer to obtain measurements of
temperature, dissolved oxygen, and salinity. An IL 175 Porto-matic
pH meter was used to measure hydrogen ion concentration.

Catch-per-unit-effort data were used to describe seasonal vari-
ations in species number, composition, abundance, and community
structure, while day-night sampling supplied information on short-
term diel variations. Catch per unit effort (C/f) for beach seines
and trawls was defined as the number of individuals per haul. For
trammel nets, C/f was defined as the number of individuals per 45-
min net set. The mean C/f by gear was calculated as follows:

$$\bar{x} = \frac{\sum_{i=1}^{n} \left[\frac{T_1}{T_2}_i \, x_i \right]}{n}$$

where

$\bar{x}$ = simple mean of C/f over catches
x_i = number collected for i^{th} catch
T_1 = unit of effort
T_2 = actual number of efforts for i^{th} catch
n = number of "observations" or catches

To test hypotheses about spatial and temporal distribution,
analysis of variance (ANOVA) was performed on response variables.
Duncan's Multiple Range procedure was used to explore the nature of

the differences indicated in each ANOVA for those variables significant at the 0.05 level.*

RESULTS AND DISCUSSION

Environmental setting

The year 1976 was extremely dry (4). Mean discharge from the Anclote River during 1976 was 1.2 m^3/s, compared with annual mean 30-yr discharge of 2.2 m^3/s (14). Largest discharges from the Anclote River occurred from May through October, with a maximum in June of 6.1 m^3/s. Dissolved oxygen and pH were variable with season and tide. Dissolved oxygen concentrations, most affected by photosynthetic activity, exhibited early morning lows and mid-afternoon highs. Relatively constant pH values were noted, with river waters slightly lower than those in the Anchorage. Salinity for the study area was high throughout the summer and during March, and lowest in December (Figure 2). Seasonal and annual changes in rainfall, river discharge, and occurrence of storms added much variability to the system.

Stations in the Anclote River were most influenced by the runoff of fresh water. As river discharge increased, salinity decreased greatly upriver at Station FS06, and to a lesser extent at stations farther downstream. Limited movement of this low salinity water was detected through the power plant to the discharge area. The decreased winter ("dry") season salinities in the Anchorage were caused by low evaporation rates and maximum subsurface discharge from the limestone aquifer.

Passage of water through the plant, either through the condensers or as dilution water, had little effect on most physical and chemical parameters (13). Mean annual increase in temperature (intake to mouth of discharge canal) was 1.2°C. Maximum observed temperature increase never exceeded 4.5°C and no thermal multiplication was observed during this study. The shallowness of the Anchorage and the resulting high insolation made differentiation between naturally heated and thermal-plume-influenced areas difficult.

Beach seining for juvenile and small fishes

Fifty-six fish species representing 30 families were seined during the January-December 1976 study period. Species occurring along the shore zone varied by station throughout the year (Table 1). The species composition of the shore zone community at Anclote typically varied depending on the unit of measure (i.e. biomass or numbers of individuals). Young-of-the-year spot (Leiostomus xanthurus) and tidewater silverside (Menidia beryllina) numerically dominated yearly beach seine collections, while silver jenny (Eucinostomus gula) and pinfish (Lagodon rhomboides) dominated total biomass collections (Table 2).

Species numbers were highest from June through October owing to migratory influx of juveniles and to a small degree to adventitious species (Figure 3). The total number of species during this

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*The statistical analyses were performed by Dr. Jim McClave and his staff, University of Florida.

period was significantly (p <0.05) greater than February, March, April, and December collections. Numbers of individuals generally followed the same seasonal pattern as species numbers except for January and February, when total numbers of individuals were significantly (p <0.05) greater than all months but June, July, and August.

Analysis of variance for beach seine sampling results indicated that mean total numbers differed significantly (p <0.05) by month and by station, but a significant month-station interaction indicated that the pattern of differences among stations was not consistent from month to month. Results were similar for mean total weights, although the trends were not quite the same as for numerical abundance (Figure 3).

Large catches of young-of-the-year fish such as spot and tidewater silverside, emphasized the use of these shore zones as nursery grounds. The schooling nature of the young of these and many other fish species accounted for the monthly fluctuations at the stations, characterized by large single catches of individual species. During winter, beach seining generally produced higher catches and greater species diversity at Station FS03, a gently sloped, shallow grass area near the discharge canal (Figure 3). Trends in species numbers and abundance at FS03 fluctuated greatly, probably in response to thermal discharge influence. Collections at FS03 during the summer were frequently lower than those at Station FS02 and other river stations, indicating that species avoided the thermal plume during this period.

Variations in species composition were least among the intake-canal station (FS02) and river stations FS04 and FS05 on either side of the intake canal. Spot, pinfish, tidewater silverside, silver jenny, spotfin mojarra (Eucinostomus argenteus), and goldspotted killifish (Floridichthys carpio) represented the more prominent species. Station FS06, located farther upriver, consistently produced lower catches and lowest species diversity. Its species composition was unique, being represented by such species as Gulf kingfish (Menticirrhus littoralis) and red drum (Sciaenops ocellata) that were not taken at any of the other stations. Since only young spotfin mojarra and tidewater silverside were taken at Station FS06 in any quantity, it appears that the majority of young of the year do not travel much beyond the mouth of the Anclote River in search of nursery grounds.

Trammel netting and trawling at the seagrass beds and discharge canal

Sixty-one species of fish representing 38 families were collected by trammel nets and bottom trawls during the 1976 study period. The occurrence of these species varied by station and method of collection (Table 3). Of the 61 species collected, only 13 were taken at all three stations, and only 12 were collected by both trammel net and bottom trawl. Owing to the unique habitat provided by the discharge canal (Station FT10), the species composition of this area was quite different from stations FT08 and FT09 located at the seagrass beds.

Sheepshead (Archosargus probatocephalus) and spotted seatrout (Cynoscion nebulosus), rarely collected at the seagrass stations,

dominated trammel net collections in the discharge canal both by number and weight (Table 4). Trammel net collections at the seagrass beds were typically light; Atlantic spadefish (Chaetodipterus faber) and Atlantic stingray (Dasyatis sabina) were collected in greatest numbers, while southern stingray (D. americana) and bonnethead shark (Sphyrna tiburo) ranked highest gravimetrically (Table 4).

Results of trammel net analysis of variance indicated no significant effects for mean total weight, while in the total species and mean total numbers models, the diel (day versus night) effect and month-station interaction were significant (p <0.05). These results implied that day/night differences existed as well as station differences, but the latter were not consistent from month to month. Duncan's multiple-range comparisons revealed that species numbers at Station FT09 were significantly (p <0.05) greater than at Station FT08 during August and October and greater than Station FT10 during October. Except for December, when mean total numbers at the discharge canal were significantly greater than at either seagrass station, there were no consistent differences among mean total numbers at the three stations, even within months. Collections at night, however, were significantly greater than day for species numbers and mean total number of individuals (Figure 4).

Pinfish overwhelmingly dominated trawl collections at the seagrass beds both by number and weight (Table 5). Trawling was most productive at the seagrass beds owing to the shelter seagrasses afforded species susceptible to this gear. Trawl collections in the discharge canal were low. The absence of vegetation and increased predator pressure in the discharge canal made this habitat unsuitable for juvenile and small fishes. Baseline data partially support this statement, as Rolfes and Mayer (10) reported that the relatively barren nature of the deep sand habitat accounted for the low number of organisms collected there (as opposed to the seagrass beds).

Interpretation of trawl analysis of variance was complicated by differences in species inhabiting the discharge canal and seagrass beds, as previously discussed. Analysis of variance for mean total numbers indicated significant differences for every effect, and revealed interactions among most of the effects. Station differences compounded the problem as the nature of these differences varied with month, diel period, and tidal cycle. Models for mean total weights indicated that among other effects, significant differences existed by month, by station, and for month-station interaction. When these effects were examined by Duncan's multiple-range comparisons, results by effect were inconsistent for abundance and biomass.

Pinfish domination of trawl collections was responsible for peak catches at Station FT09 during December and especially October, contributing to significantly (p <0.05) greater biomass and numbers of individuals during October than in all other months. Biomass, being affected by number and size of individuals, was influenced by low catches of larger species, such as ladyfish (Elops saurus), which partly explained the disparities in seasonal trends between numbers and biomass (Figure 5). Overall, the mean total number of individuals and species collected at Station FT10 by trawl was significantly (p <0.05) less than the total from the other stations, and mean total weights at Station FT09 tended to be higher than

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either FT08 or FT10. Night collections produced significantly greater mean total numbers and biomass at all stations. Species numbers were significantly (p   0.05) higher during June and October than for trawl collections during other months. Shifts in adventitious species, possibly in response to higher temperatures during August, were responsible for the drop in numbers of individuals and species during midsummer (Figure 5).

Though the species composition and size of individuals collected by trammel net and trawl were considerably different, both gear revealed similar seasonal trends that were related to well-documented inshore (spring) and offshore (winter) migrations of Gulf of Mexico fishes (9, 11). The abundance of fishes inhabiting the seagrass beds was generally low during winter, increased nearly three-fold during spring, decreased into the summer, and was highest in the fall owing to increased concentrations of resident species (Figures 4 and 5). Seasonal trends in species numbers revealed lows during winter and summer, as highest values were recorded during spring owing to the migratory influx of offshore overwintering species. The increased variety of species taken during fall was attributed to local shifts in resident species and an influx of adventitious species, probably in response to the warmer waters in the vicinity of the power plant. Abundance and number of species in the discharge canal remained low during all but the colder months.

Seagrass stations FT08 and FT09 generally exhibited comparable catches and species diversity in all but the colder months. During October, the number of species taken in trammel net collections at Station FT09 was significantly greater than at Station FT08, as were trawl collections for total fish number and biomass. Station FT09 is located in a shallow grass area approximately 0.8 km from the discharge canal and is thereby influenced to a greater extent by the effluent. With the exception of discharge canal Station FT10, mean temperatures at Station FT09 were significantly higher than at all other stations. Station FT08, on the southeastern shore of Rabbit Key and just south of the Anclote River channel, is protected by the intervening deeper waters of the Anchorage, which help buffer the effects of thermal loading.

It is likely that increased water temperatures in the immediate vicinity of the power plant delayed offshore winter migrations of many species to deeper and warmer waters. Apparently, the concentration or overwintering of fish in deeper water areas is not fixed, and fish may accept an increase in water temperature instead of depth (8). As temperatures continued to drop with the approach of winter, however, fish sought deeper waters to avoid the temperature fluctuations characteristic of inshore shallow areas such as Station FT09. It was during this period that migrating fishes found their way into the discharge and intake canals, resulting in increased impingement rates (12) and significantly greater trammel net collections in the discharge.

Comparisons with preoperational data, though limited, revealed similar seasonal trends, with number of individuals and number of species declining during winter (3, 10). However, noticeable decreases in summer abundance and species numbers as observed during 1976 were not observed during baseline surveys. In fact, Baird et

al. (3) reported that the maximum number of fish and species per
unit effort was taken in trawls during August (summer).

Seasonal patterns of the present study closely resemble those
reported by Grimes and Mountain (5) at FPC's Crystal River power fa-
cility. These authors reported a seasonal alternation of diversity
and abundance associated with the thermal effluent that was charac-
terized by winter increases and summer decreases. Water quality
data do not substantiate any significant increases in temperature or
other profound parameter changes from those measured during preopera-
tional Anclote surveys. Admittedly, seasonal patterns of fish dis-
tribution in the estuary may be incompletely defined owing to short-
comings in sampling frequency during present and past surveys (3).
Thus, it could not be determined whether seasonal patterns observed
during 1976 were short-term movements or redistribution of a more
permanent nature.

In summary, thermal discharges from the Anclote Power Plant
had a very localized effect on the Gulf fishes of the Anchorage.
Natural abundance and diversity were only slightly altered during
the colder months, and avoidance of the thermal plume during the
hottest months of summer was evident. Since fish are mobile and can
generally avoid unfavorable conditions, the possibility of fish
kills is low in all but the coldest months, when entrapment in the
thermal plume combined with sudden drops in temperature could pose
problems. Since the estuary in the vicinity of the Anclote Power
Plant is an open system, the abundance of fishes in the area is in-
fluenced by the migration of individuals into and out of the estu-
ary. Consequently, any available habitat temporarily vacated be-
cause of transient man-induced effects at Anclote should be filled
by migrant individuals from adjacent areas.

SUMMARY AND CONCLUSIONS

To evaluate the effects of thermal discharges from the Anclote
Power Plant, near-field studies of fishes were conducted with beach
seines, trawls, and trammel nets. Studies conducted during 1976 com-
pared data from stations removed from potential plant effects with
data collected from stations adjacent to plant intake and discharge.
Comparisons were also made with preoperational information collected
in the vicinity of the Anclote site.

Beach seining exhibited large catches of young-of-the-year
fish emphasizing the importance of the estuary's shore zones as nurs-
ery grounds. Young-of-the-year spot, tidewater silverside, silver
jenny, and pinfish dominated yearly beach seine collections. Vari-
ations in species composition were least among the intake canal sta-
tion and the two river stations on either side of the canal. Collec-
tions at the uppermost river station consistently produced low
catches and lowest species diversity, indicating that most young
fish do not travel that far upstream in search of nursery grounds.

Collections from the shore zone generally exhibited a marked
seasonal pattern related to the inshore-offshore migration of non-
resident species and the nursery function of the estuary. Abundance
and species diversiy were generally greatest during the warmer
months, as reported during preoperational data surveys. Fishes at
Station FS03, most affected by the thermal plume, were more abundant

during the coldest months and exhibited decreases during the hottest periods. Since the shore zone of the Anclote Anchorage is normally sparse during winter, it is concluded that the reversal of normal abundance was in response to the thermal effluent. Likewise, evidence of decreased abundance and diversity at shallow, thermally influenced areas during summer months indicated avoidance of the heated effluent.

Comparable trammel net and trawl sampling conducted in the discharge canal revealed few species similarities when compared to collections at the seagrass beds. Abundance and numbers of species remained low during all but the colder months, with collections dominated by sheepshead, a species rarely collected on the seagrass beds. The seagrass stations generally exhibited comparable catches and species diversity except for the colder months, when larger collections at Station FT09 were attributed to the thermal effluent. Pinfish was the dominant species at the seagrass beds.

Comparisons with baseline data revealed similar seasonal trends at the seagrass beds, with increased spring and fall collections. However, noticeable decreases in summer abundance and species numbers as observed during 1976 were not recorded during baseline surveys.

In concluding, the thermal discharge from the Anclote Power Plant had a very localized effect on the fishes inhabiting the Anchorage. Natural abundance and diversity at affected stations increased during the colder months and decreased during warmer months, but dominant seasonal trends were related to well-documented inshore (spring) and offshore (winter) migrations.

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FISH SAMPLING STATIONS
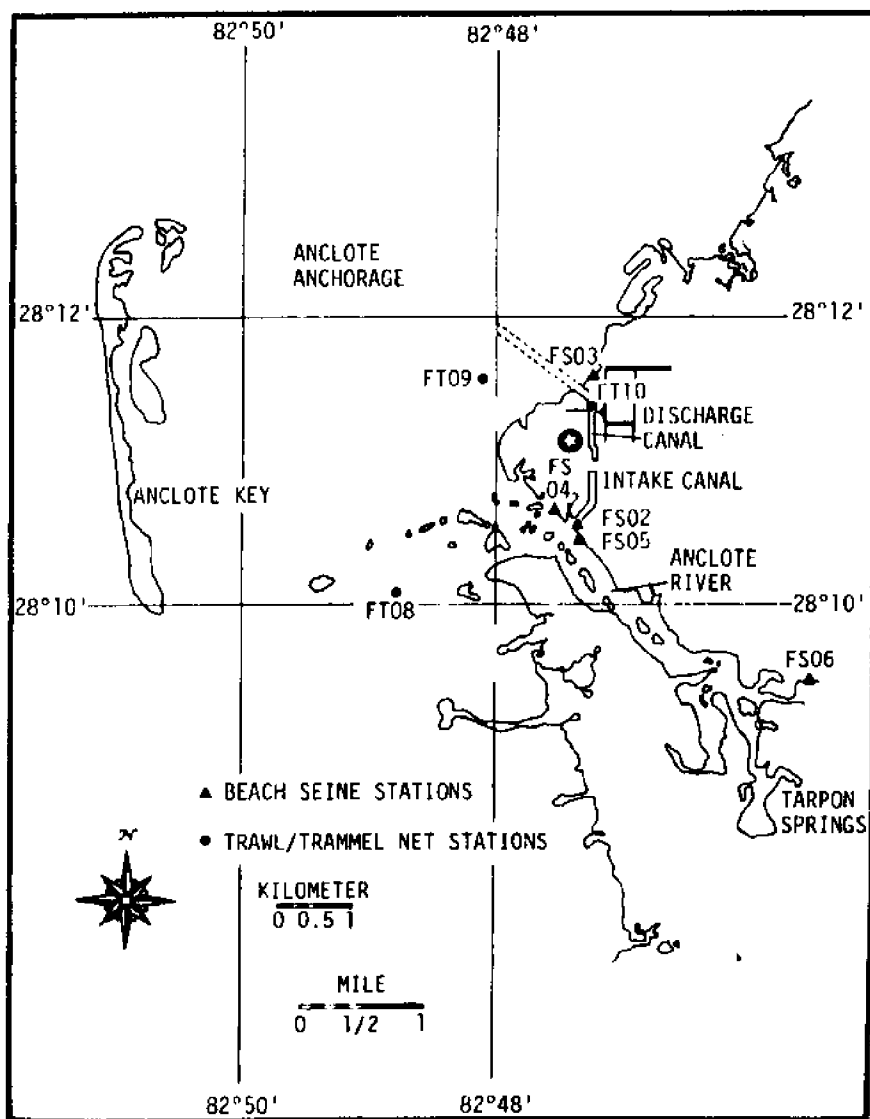
Figure 1. Fish sampling stations at Anclote Power Plant site, 1976.
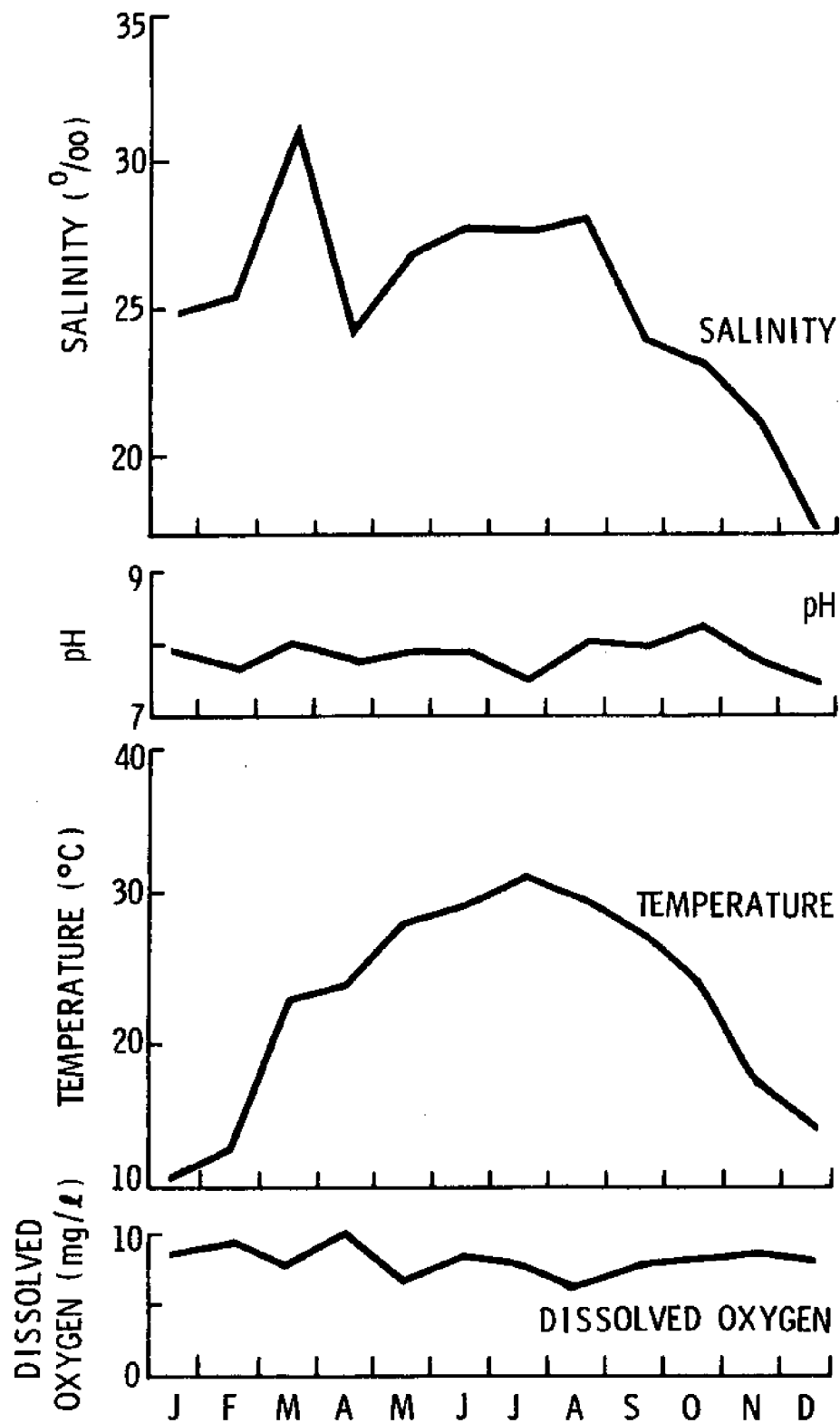
Figure 2. Monthly means for salinity, temperature, dissolved
oxygen and pH, Anclote site, 1976.
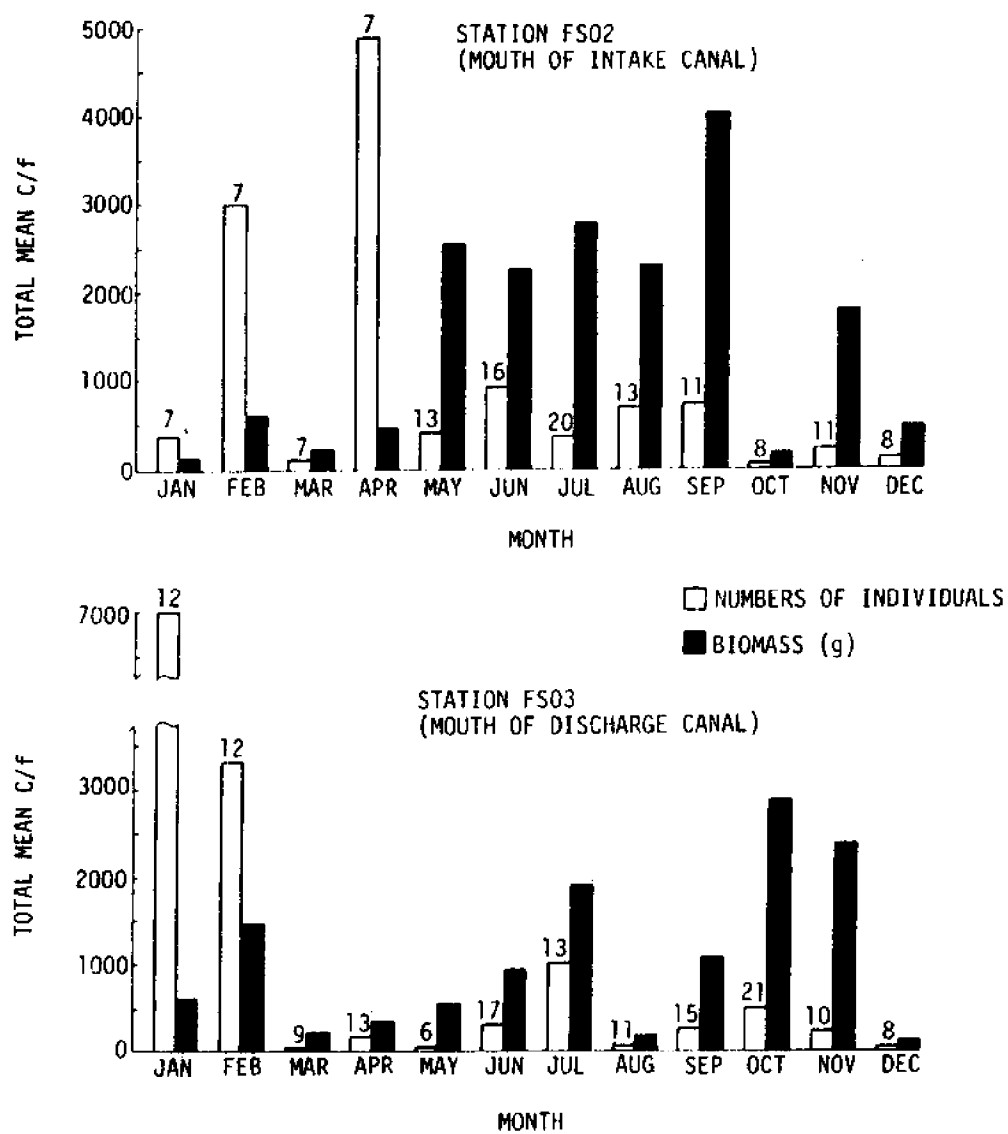
Figure 3. Total mean catch per effort (C/f) for fish collected
           by beach seine, Anclote site, 1976 (Numbers above
           bars represent numbers of species).
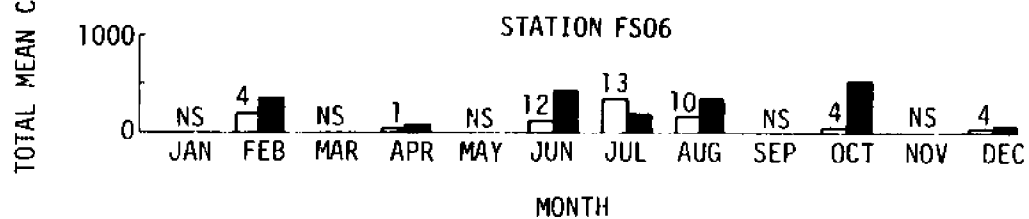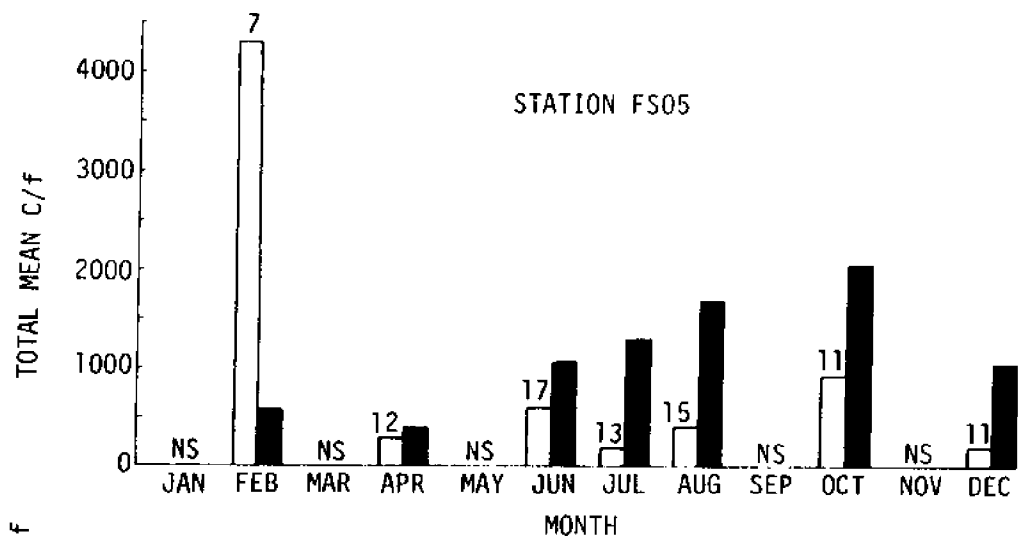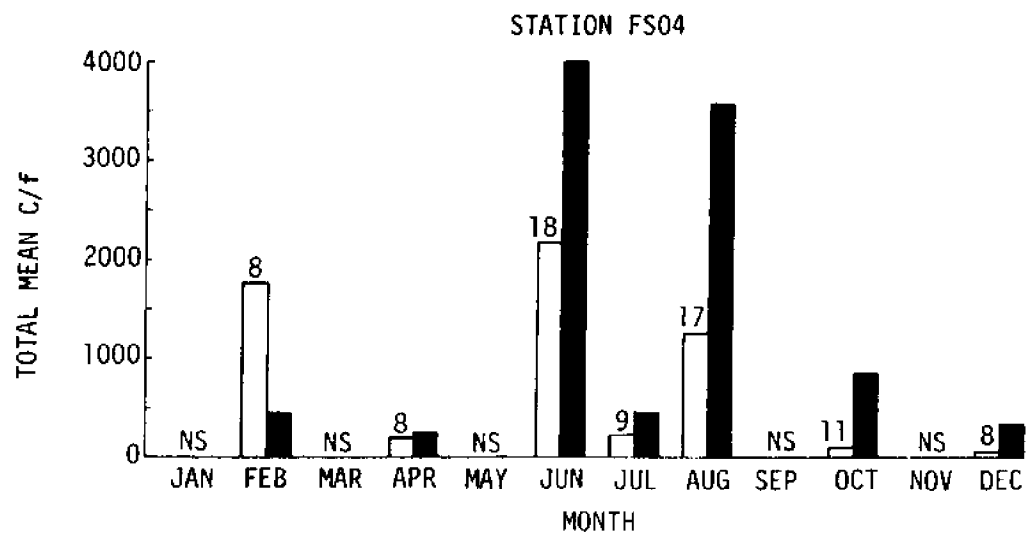
Figure 3. (Contd)

NS = No Sample
NC = No Catch

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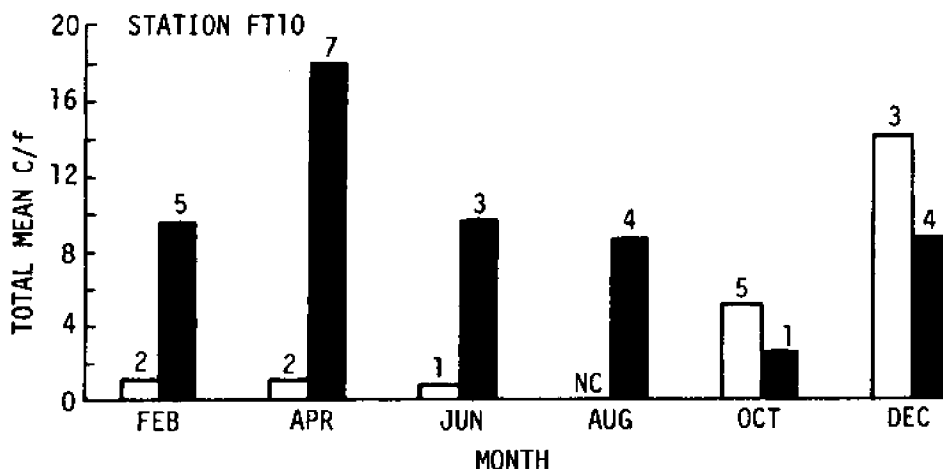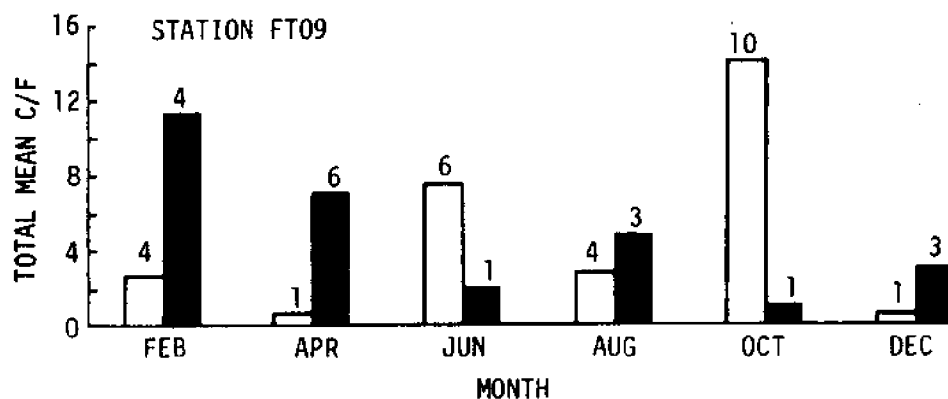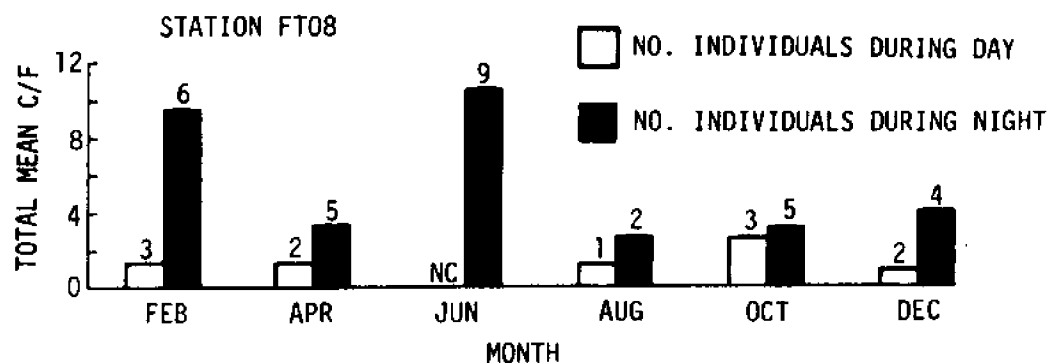
Figure 4. Total mean catch per effort (C/f) for fish collected by trammel net, Anclote site, 1976 (Numbers above bars represent numbers of species).
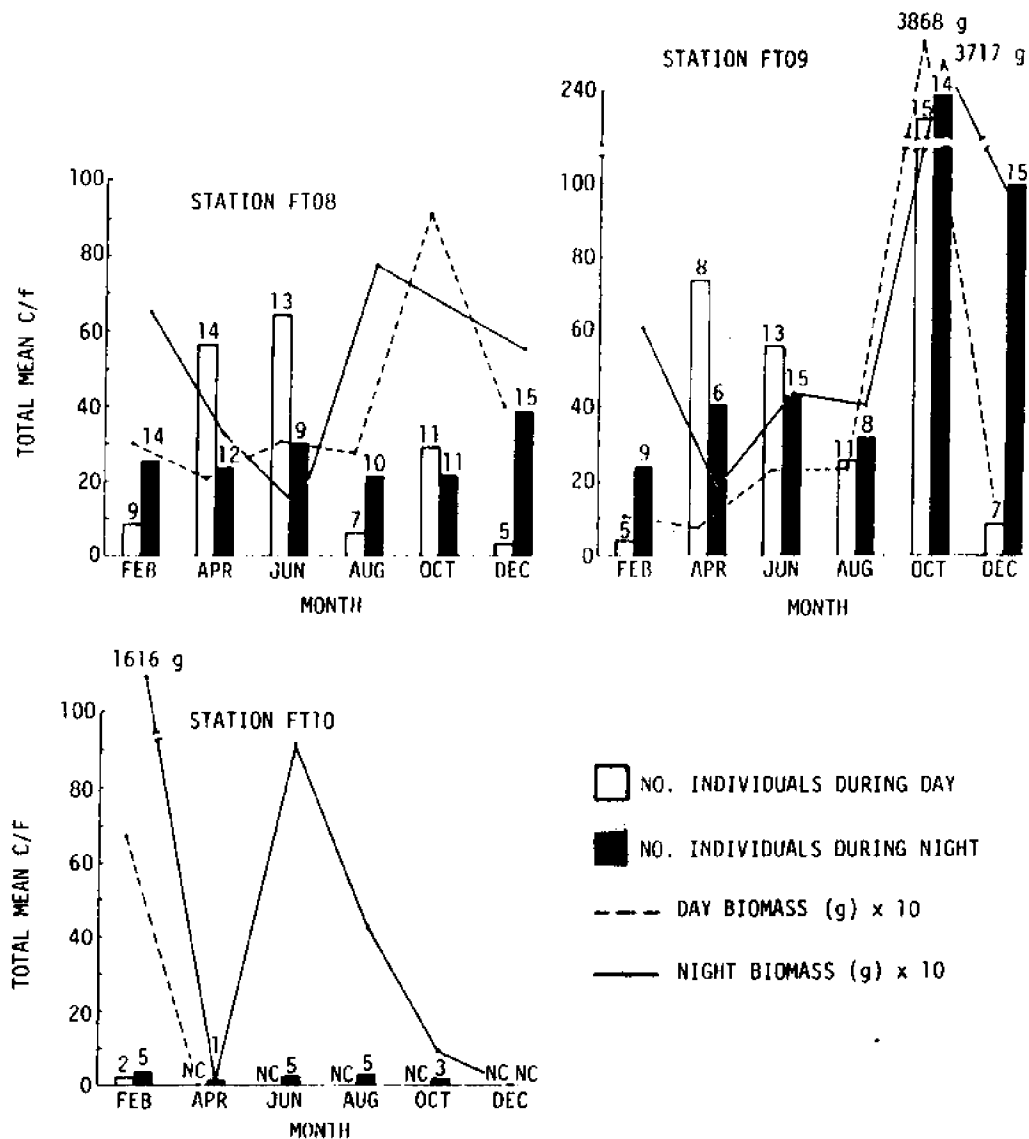
Figure 5. Total mean catch per effort (C/f) of fish collected by trawl, Anclote site, 1976 (Numbers above bars represent numbers of species).

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Table I. Fishes taken during seining, Anclote site, January–December 1976 (Numbers in columns correspond to station designations).

| Scientific Name	Common Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Dasyatis sabina	Atlantic stingray						4	2					
Harengula pensacolae	Scaled sardine						2					3	
Anchoa mitchilli	Bay anchovy		4,5		4		3,4	2,6	4		2,3,4,5,6		
Synodus foetens	Inshore lizardfish		6		2		4,5.		4,5	2	2		
Arius felis	Sea catfish												
Hyporhamphus unifasciatus	Halfbeak				5	2	2,3,5	2			3	2	
Strongylura marina	Atlantic needlefish		3,4	2	2,3,4,5		2	2			2,3,4,5		
Strongylura notata	Redfin needlefish	3	2,3,4	3	3	2,3	2,3,4,5	2,3,4,5,6	2,3,4,5	2,3	2,3,4,5	2,3	2,3,4,5,6
Strongylura timucu	Timucu					2,3	2,3,4,5,6	2,3,4,5,6	2,3,4,5,6	2,3	3,4,5,6	2,3	2,3,4,5,6
Cyprinodontidae	Killifish				2								
Cyprinodon variegatus	Sheepshead minnow		3					6	3,4,6				4
Floridichthys carpio	Goldspotted killifish	2,3	2,3,4,5	2,3	3,5	2	2,3,4,5	2,3,4,5,6	2,3,4,5,6	2,3	3,5,6	2,3	2,3,4,6
Fundulus confluentus	Marsh killifish	2	2								5		
Fundulus grandis	Gulf killifish												
Fundulus similis	Longnose killifish	2	5	2	2,3		3,4,6	6	3,4,5,6	3	3,4,5	3	2,3,5
Lucania parva	Rainwater killifish	3	2,3	2,3	3,4	2	2,4,6	3,6	2,3,4,6	3	3		2
Poecilia latipinna	Sailfin molly							6	4,5				
Menidia beryllina	Tidewater silverside	2,3	2,3,4,5	3	2,3,4,5	2,3	2,3,4,5,6	3,6	2,4,5,6	3	3,5		2,5
Hippocampus zosterae	Dwarf seahorse	2											
Syngnathus floridae	Dusky pipefish						4,5	3					
Syngnathus louisianae	Chain pipefish				3								
Syngnathus scovelli	Gulf pipefish				3,4,5		2,4,5,6	2,4,5	2,4,5		3,4	2	
Centropomus undecimalis	Snook					2		2					
Caranx hippos	Crevalle jack							3					
Chloroscombrus chrysurus	Atlantic bumper							3			3		
Oligoplites saurus	Leatherjacket						3,5	2,3,5	3,4,6	2,3			
Trachinotus falcatus	Permit							2		2,3	2,3	2	
Lutjanus griseus	Gray snapper									3	3		

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Table 1. (Contd)

Scientific Name	Common Name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Gerreidae	Mojarras												
Diapterus plumieri	Striped mojarra						6	2,5			3		
Eucinostomus argenteus	Spotfin mojarra	3	3,6	3	3,6	2	2,3,4,5	2,3,5,6	2,3,4,5,6	2,3	2,3,4,5,6	2,3	3,4,5,6
Eucinostomus gula	Silver jenny				5	2,3	2,4,5	2,3,4,5	2,3,4,5,6	2,3	3	2,3	2,5
Orthopristis chrysoptera	Pigfish						3,4	2,4,6	2,5				
Archosargus probatocephalus	Sheepshead							2,4,6	4,5	3	3		
Lagodon rhomboides	Pinfish	2,3	2,3,4,5,6	2,3	2,3,4,5	2,3	2,3,4,5,6	2,3,4,5	2,4,5,6	2,3	3,4,5	2,3	2,3,5
Bairdiella chrysoura	Silver perch						2,3,4,5	2,5	2,5		4		
Cynoscion spp.	Seatrouts	3											
Cynoscion nebulosus	Spotted seatrout	2,3	2,3,4,5				4,5	2	2,5		3		
Leiostomus xanthurus	Spot	2,3	2,3,4,5	2	3,5	2	2,3,4,5	3					
Menticirrhus littoralis	Gulf kingfish	3					6						
Micropogon undulatus	Atlantic croaker		6										
Sciaenops ocellata	Red drum												
Chaetodipterus faber	Atlantic spadefish							5					
Mugilidae	Mullets	3	3						3		3		3
Mugil cephalus	Striped mullet		3				3	6					
Mugil trichodon	Fantail mullet		3	3			3,6	3		3	3,5	3	5
Astroscopus y-graecum	Southern stargazer							2					
Chasmodes saburrae	Florida blenny						2						
Hypsoblennius hentzi	Feather blenny												
Gobiosoma robustum	Code goby				5								
Microgobius gulosus	Clown goby				3,4		2,6	6					
Prionotus scitulus	Leopard searobin										2		
Prionotus tribulus	Bighead searobin										2		
Paralichthys albigutta	Gulf flounder			2	5								
Achirus lineatus	Lined sole									2			
Trinectes maculatus	Hogchoker								6				
Symphurus plagiusa	Blackcheek tonguefish								4				
Monacanthus hispidus	Planehead filefish				5		3						
Sphoeroides nephelus	Southern puffer	3	5	3	2,3,4,5	2,3	2,3,4,5	2,3	2	2	2,3,4,5	2,3	3,4,5
Chilomycterus schoepfi	Striped burrfish										4		
	Total No. Species	14	18	12	21	13	29	33	23	18	28	13	13
	No. Samples	7	10	4	12	4	12	12	12	4	12	4	10

Table 2. Species composition of dominant fishes collected by beach seine at Anclote, 1976.

INTAKE STATION FS02
(Number samples = 24)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Tidewater silverside	10208	43.6	Silver jenny	11296.0	31.6
Spot	5158	22.0	Pinfish	7196.5	20.2
Pinfish	3289	14.1	Redfin needlefish	4455.1	12.5
Silver jenny	2444	10.4	Spotfin mojarra	4009.8	11.2
Spotfin mojarra	1277	5.4	Tidewater silverside	1600.8	4.5
Goldspotted killifish	368	1.6	Goldspotted killifish	1279.9	3.6
Redfin neeldefish	280	1.2	Timucu	1278.4	3.6
Longnose killifish	78	0.3	Spot	824.0	2.3
Silver perch	55	0.2	Striped mojarra	354.6	1.0
Gulf pipefish	46	0.2	Longnose killifish	166.2	0.5
Other	194	0.8	Other	3234.8	9.0
Total	23397		Total	35696.1	

DISCHARGE STATION FS03
(Number samples = 36)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Spot	18543	60.4	Tidewater silverside	6368.5	17.0
Tidewater silverside	4171	13.6	Silver jenny	5848.2	15.8
Mullet spp.	1976	6.4	Fantail mullet	5632.9	15.1
Silver jenny	1862	6.1	Pinfish	4024.9	10.8
Goldspotted killifish	1420	4.6	Redfin needlefish	3888.7	10.4
Pinfish	1022	3.3	Goldspotted killifish	2938.7	7.9
Faintail mullet	376	1.2	Spot	1964.8	5.2
Spotfin mojarra	365	1.2	Spotfin mojarra	1630.6	4.4
Redfin needlefish	263	0.8	Timucu	1277.3	3.4
Longnose killifish	189	0.6	Longnose killifish	873.5	2.3
Other	538	1.8	Other	2895.1	7.7
Total	30725		Total	37393.2	

RIVER STATIONS FS04-FS06
(Number samples = 36)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Spot	10782	38.5	Pinfish	10918.0	26.6
Pinfish	4481	16.0	Silver jenny	9368.2	22.9
Silver jenny	4317	15.4	Tidewater silverside	4451.5	10.9
Tidewater silverside	3423	12.2	Spotfin mojarra	3578.4	8.7
Spotfin mojarra	1835	6.5	Timucu	2550.8	6.2
Goldspotted killifish	940	3.4	Goldspotted killifish	2117.4	5.2
Mojarra spp.	728	2.6	Spot	1281.8	3.1
Pigfish	262	0.9	Pigfish	853.0	2.1
Gulf pipefish	244	0.9	Redfin needlefish	809.7	2.0
*Red drum	156	0.6	Longnose killifish	705.7	1.7
Other	859	3.0	Other	4364.1	10.6
Total	28027		Total	40998.6	

*Taken only at Station FS06

Table 3. Fishes taken during trammel net and trawl sampling, Anclote site, 1976 (Numbers in columns represent station designations).

Scientific Name	Common Name	February Trawl	February Trammel	April Trawl	April Trammel	June Trawl	June Trammel	August Trawl	August Trammel	October Trawl	October Trammel	December Trawl	December Trammel
Carcharhinus leucas	Bull shark						9						10
Carcharhinus limbatus	Blacktip shark		8		9								8
Mustelus canis	Smooth dogfish												8
Sphyrna tiburo	Bonnethead shark												
Rhinobatos lentiginosus	Atlantic guitarfish				8,9		8,9		8,9		8,9		
Dasyatis americana	Southern stingray		8		8,9		8		8,9		8,9	8	8
Dasyatis sabina	Atlantic stingray		8,10		9,10	8,10	8,9,10	8,10	8,9,10		8,9	8	8
Dasyatis sayi	Bluntnose stingray						9		9				
Gymnura micrura	Smooth butterfly ray				8		8				9		8
Rhinoptera bonasus	Cownose ray		8								9		8
Lepisosteus spatula	Alligator gar		8								9		
Elops saurus	Ladyfish	10	10										
Brevoortia sp.	Menhaden		8										
Brevoortia smithi	Yellowfin menhaden										8		8
Synodus foetens	Inshore lizardfish	8,9						9			8,9		
Bagre marinus	Gafftopsail catfish				10		8	10	8,9	9	8,9		8,9
Arius felis	Sea catfish	8,9	9	8,9	8,9	8	8	8,9		8,9	8,9	8	
Opsanus beta	Gulf toadfish	8		9,10	10	10						8	
Urophycis floridanus	Southern hake	8,9		8	8,9	8,9						8	
Ophidion holbrooki	Bank cusk-eel							9					
Lucania parva	Rainwater killifish	8		8									
Hippocampus erectus	Lined seahorse	8		8									
Hippocampus zosterae	Dwarf seahorse	8		8	8								
Micrognathus crinigerus	Fringed pipefish	9		8,9	8,9			9				8	
Syngnathus floridae	Dusky pipefish	8,9										8,9	8,9
Syngnathus louisianae	Chain pipefish											8,9	8,9
Syngnathus scovelli	Gulf pipefish	8,9		8,9	8,9							8,9	8,9
Centropristis melana	Southern sea bass	8											
Diplectrum formosum	Sand perch	8		8									
Epinephelus itajara	Jewfish	10		10									
Pomatomus saltatrix	Bluefish	10	10										

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Table 3. (Contd)

Scientific Name	Common Name	February Trawl	February Trammel	April Trawl	April Trammel	June Trawl	June Trammel	August Trawl	August Trammel	October Trawl	October Trammel	December Trawl	December Trammel
Caranx hippos	Crevalle jack		9					9					
Lutjanus griseus	Gray snapper												
Eucinostomus argenteus	Spotfin mojarra												
Eucinostomus gula	Silver jenny			8,9				8,9		8,9,10		8,9	
Haemulon plumieri	White grunt	8		9						8,9,10		8,9	
Orthopristis chrysoptera	Pigfish	8		8,9		8,9		8,9		8,9,10		8,9	
Archosargus probatocephalus	Sheepshead	10	10	9,10	10	8	10	9,10	10	8,9	9,10	9	10
Calamus arctifrons	Grass porgy			8						8			
Diplodus holbrooki	Spottail pinfish	8		8						9		8,9	
Lagodon rhomboides	Pinfish	8,9		8,9		8,9		8,9		8,9		8,9	
Sciaenidae	Drums	9		9									
Bairdiella chrysura	Silver perch	9		8,9		8,9		8,9,10		8,9		8,9	
Cynoscion nebulosus	Spotted seatrout		10	8,9			10	9	9	9			10
Leiostomus xanthurus	Spot			10						10			
Pogonias cromis	Black drum		10	10			10		10		9,10	10	
Chaetodipterus faber	Atlantic spadefish	9,10	9,10	10	9	8,9	8,9,10		9,10	9	9,10	10	9,10
Lachnolaimus maximus	Hogfish	8		8,9				8					
Nicholsina usta	Emerald parrotfish	8		8									
Mugil cephalus	Striped mullet	10				8							
Sphyraena borealis	Northern sennet			8									
Hypsoblennius hentzi	Feather blenny			9									
Gobiosoma robustum	Code goby			9									
Scorpaena brasiliensis	Barbfish			8									
Prionotus scitulus	Leopard searobin	8								8			
Paralichthys albigutta	Gulf flounder	8	8	8,9	10		8	8,9,10		9	8,9,10	8,9	
Ancylopsetta quadrocellata	Ocellated flounder		9	9				9		9		9	
Trinectes maculatus	Hogchoker		9							9			
Monacanthus ciliatus	Fringed filefish	8		8,9		8,9				8,9		8,9	
Monacanthus hispidus	Planehead filefish	8		8,9		8,9		8		8,9		8,9	
Lactophrys quadricornis	Scrawled cowfish	8,9,10	8,9	9	8,10	8,9	9	9		8,9	9,10	9	
Sphoeroides nephelus	Southern puffer	9	8,9	9	8,9	9		9		8,9		8,9	
Chilomycterus schoepfi	Striped burrfish	8,9	8,9	8,9	8,9	8,9	8,9	8,9	9	8,9		8,9	
Total No. Species		23	17	23	13	24	14	18	9	24	12	18	13

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Table 4. Species composition of dominant fishes collected by trammel net at Anclote, 1976

SEAGRASS BEDS
(Number samples = 48)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Atlantic spadefish	36	18.5	Southern stingray	36945.6	21.1
Atlantic stingray	27	13.8	Bonnethead shark	33374.1	19.0
Bonnethead shark	23	11.8	Cownose ray	18088.8	10.3
Sea catfish	18	9.2	Atlantic stingray	12753.8	7.3
Striped burrfish	15	7.7	Bluntnose stingray	11360.0	6.5
Southern stingray	11	5.6	Smooth butterfly ray	9070.0	5.2
Gulf flounder	10	5.1	Sea catfish	8566.7	4.9
Menhaden	9	4.6	Smooth dogfish	7498.4	4.3
Scrawled cowfish	9	4.6	Alligator gar	6702.4	3.8
Atlantic guitarfish	6	3.1	Atlantic spadefish	6378.2	3.6
Other	31	16.0	Other	24509.5	14.0
Total	195		Total	175247.5	

DISCHARGE CANAL
(Number samples = 24)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Sheepshead	78	50.0	Spotted seatrout	46604.9	41.4
Spotted seatrout	37	23.7	Sheepshead	29839.0	26.5
Atlantic spadefish	13	8.3	Southern stingray	15989.4	14.2
Black drum	8	5.2	Black drum	4311.0	3.8
Atlantic stingray	7	4.5	Atlantic spadefish	4150.6	3.7
Scrawled cowfish	4	2.6	Atlantic stingray	3569.3	3.2
Southern stingray	3	1.9	Bull shark	3558.8	3.2
Gulf flounder	2	1.3	Scrawled cowfish	1192.8	1.0
Bull shark	1	0.6	Gulf flounder	1022.4	0.9
Bluefish	1	0.6	Bluefish	1008.6	0.9
Other	2	1.3	Other	1409.4	1.2
Total	156		Total	112674.6	

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Table 5. Species composition of dominant fishes collected by trawl at
Anclote, 1976

SEAGRASS BEDS
(Number samples = 48)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Pinfish	3533	75.4	Pinfish	38230.9	58.2
Silver perch	409	8.7	Striped burrfish	7273.6	11.1
Pigfish	306	6.5	Pigfish	5525.6	8.4
Striped burrfish	72	1.5	Silver perch	3166.3	4.8
Silver jenny	55	1.1	Scrawled cowfish	2668.2	4.1
Gulf toadfish	36	0.8	Gulf toadfish	1766.0	2.7
Dusky pipefish	32	0.7	Atlantic stingray	1470.2	2.2
Gulf pipefish	25	0.5	Southern puffer	1098.1	1.7
Scrawled cowfish	24	0.5	Gulf flounder	641.2	1.0
Fringed filefish	21	0.4	Sea catfish	446.8	0.7
Other	169	3.7	Other	3379.1	5.1
Total	4682		Total	65666.0	

DISCHARGE CANAL
(Number samples = 24)

Numerical			Gravimetric		
Species	No.	%	Species	Wt (g)	%
Sheepshead	12	30.8	Ladyfish	4537.2	30.0
Southern hake	6	15.4	Sheepshead	3259.1	21.5
Atlantic spadefish	3	7.7	Jewfish	1760.8	11.6
Silver jenny	2	5.1	Atlantic spadefish	1412.2	9.3
Ladyfish	2	5.1	Bluefish	1400.0	9.2
Bluefish	2	5.1	Striped mullet	656.1	4.3
Spot	2	5.1	Black drum	482.8	3.2
Black drum	1	2.6	Spot	470.1	3.1
Striped mullet	1	2.6	Atlantic stingray	312.4	2.1
Jewfish	1	2.6	Scrawled cowfish	282.5	1.9
Other	7	17.9	Other	558.1	3.8
Total	39		Total	15131.3	

INSTRUMENTAL ANALYSIS OF VOLATILES IN SEAFOOD

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For many years researchers have worked to develop objective methodology for evaluating flavor and flavor stability of food products. Practical methods, however, have not readily emerged, and food processors have relied on sensory evaluation to assess the flavor quality of their product. Such evaluation is tedious, time-consuming, and often unreliable. Recent progress at Southern Regional Research Center in the development of direct gas chromatographic techniques for analyzing food volatiles associated with flavor (1, 2) has prompted one major food processing corporation to adapt this novel method to measure flavor quality and shelf life stability of salad and cooking oils.

Seafood is a perishable product that must be refrigerated immediately after being taken and must subsequently be cleaned and frozen as quickly as possible to minimize decomposition. Since decomposition is generally associated with the development of undesirable odors and off-flavors, it would appear reasonable to monitor the volatile components in a product to identify the decompositional changes that occur during handling, processing, and storage. This paper reports on progress in developing a rapid, unconventional, gas chromatographic technique for the elution and resolution of volatiles that may be associated with seafood decomposition.

MATERIALS

Silicone O-rings obtained from Teklab, Inc., Baton Rouge, La., were conditioned for two hours at 200°C. Sandwich-type silicone septa from Hamilton Co., Reno, Nevada, were immersed in chloroform for 15 minutes, rinsed with chloroform, air-dried, and then conditioned for two hours at 200°C. Pyrex brand glass wool, manufactured by Corning Glass Works, Corning, New York, was heated at 200°C. for approximately 16 hours to remove volatiles. Liners, 10 X 84 mm, were cut from borosilicate glass tubing. Certified A.C.S. potassium carbonate (anhydrous) was obtained from Fisher

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Scientific Company. Porapak Q, 80/100 mesh, was obtained from Waters Associates, Framingham, Mass. Commercial and experimental seafood products were used.

SAMPLE PREPARATION

Approximately 200 mg of glass wool was placed at one end of a gas chromatographic injection-port glass liner. Between 100 and 300 mg of potassium carbonate was added on the glass wool, and this was capped with about 50 mg additional glass wool. A quantity of 50 to 300 mg of seafood tissue or oyster liquor was added to the tube contents, and then capped with about 50 mg additional glass wool.

GAS CHROMATOGRAPHY PROCEDURE

A Tracor 220 gas chromatograph (GC) equipped with dual flame ionization detectors, a Hewlett-Packard 3380A integrator, and a Westronics MT 22 recorder were used. The construction of the GC has been described previously (2, 3). The column was a 1/8 in. X 6 ft. stainless steel U tube packed with Porapak Q, 80/100 mesh. Nitrogen was used as a carrier gas, with a pressure of 60 psi on the regulator. Rotameter settings were maintained at 40 ml/min. The pressures and rotameter settings for hydrogen and air were 30 psi, 45 ml/min, and 50 psi, 560 ml/min, respectively.

The glass liner containing the sample was placed in the heated injection port of the GC at 120°C. for 10 minutes to steam-distill the volatiles and transfer them onto the head of the GC column. The potassium carbonate in the liner trapped excess moisture and organic acids which could adversely affect the GC column temperature was quickly raised to 70°C., then programmed to rise 5°C/min to a final holding temperature of 215°C. This temperature was maintained until elution of volatiles was complete. A typical analysis was accomplished in a total of 70 to 80 minutes.

RESULTS AND DISCUSSION

Profiles of volatiles were obtained from different types of seafood samples, held under various conditions of storage. The profiles appear to be generally similar for the different types of seafood; however, in all instances, the volatile component peaks become more intense with prolonged storage. This effect is particularly apparent in the analysis of raw oysters. Figure 1 shows how the concentration of volatiles increases rapidly in oysters during storage. The profile of volatiles obtained from the aqueous solution containing oysters stored on ice for six hours (chromatogram 1) shows only four well-defined peaks, and these occur in moderate concentration. In contrast (chromatogram 2), when the liquid was stored at 25°C. for 16 hours, the number of well-defined peaks more than doubled, and the intensity of each vastly increased. Similarly, storage of the oyster liquor under refrigeration for 16 days (chromatogram 3) shows a multitude of peaks of high intensity, with a vast buildup of unresolved material appearing during the 35 to 55 minute

retention time. Although the profiles of volatiles and sensory data have not yet been correlated for these products, previous works (2-4) demonstrated that an increase in the number and intensity of volatile peaks during storage is directly related to flavor deterioration.

Figure 2 shows the profile of volatiles obtained for trout stored at $-10^{\circ}C$. for 2, 11, and 25 months. Although refrigeration temperature remained constant, it is apparent that changes occur in the trout samples with storage. The trout stored for two months (chromatogram 1) reveals only four major volatile peaks of average intensity. As the storage time increased to 11 months (chromatogram 2), many of these peaks materially increased in size, and several new peaks, not present in the fresher sample, have emerged. Storage of the trout for 25 months (chromatogram 3) results in a profile of volatiles with still more peaks, and a major increase in peak intensities. In other products studied, such an increase in the number and intensity of volatile peaks has been directly correlated with flavor deterioration occurring on storage.

CONCLUSIONS

These investigations indicate that volatiles from less than 300 mg of seafood tissue or aqueous solution can be effectively analyzed by direct gas chromatography, with no prior enrichment of sample volatiles. This rapid, unconventional approach eliminates the tedious and time-consuming extractions and distillations that precede gas chromatographic analysis. With this novel approach it should be possible to develop practical gas chromatographic techniques to measure flavor changes and decomposition of seafood products that occur during handling, processing, and storage.

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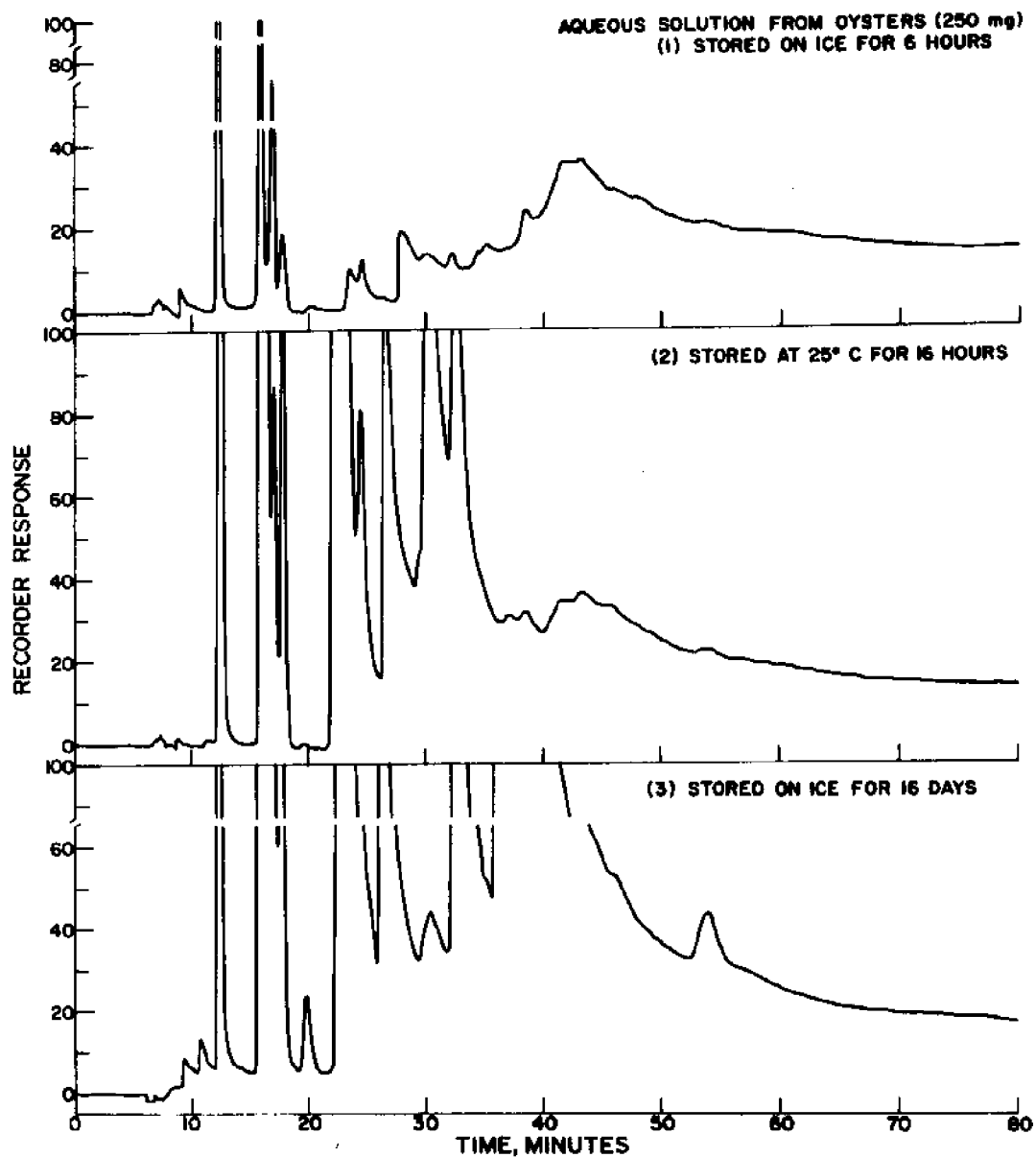
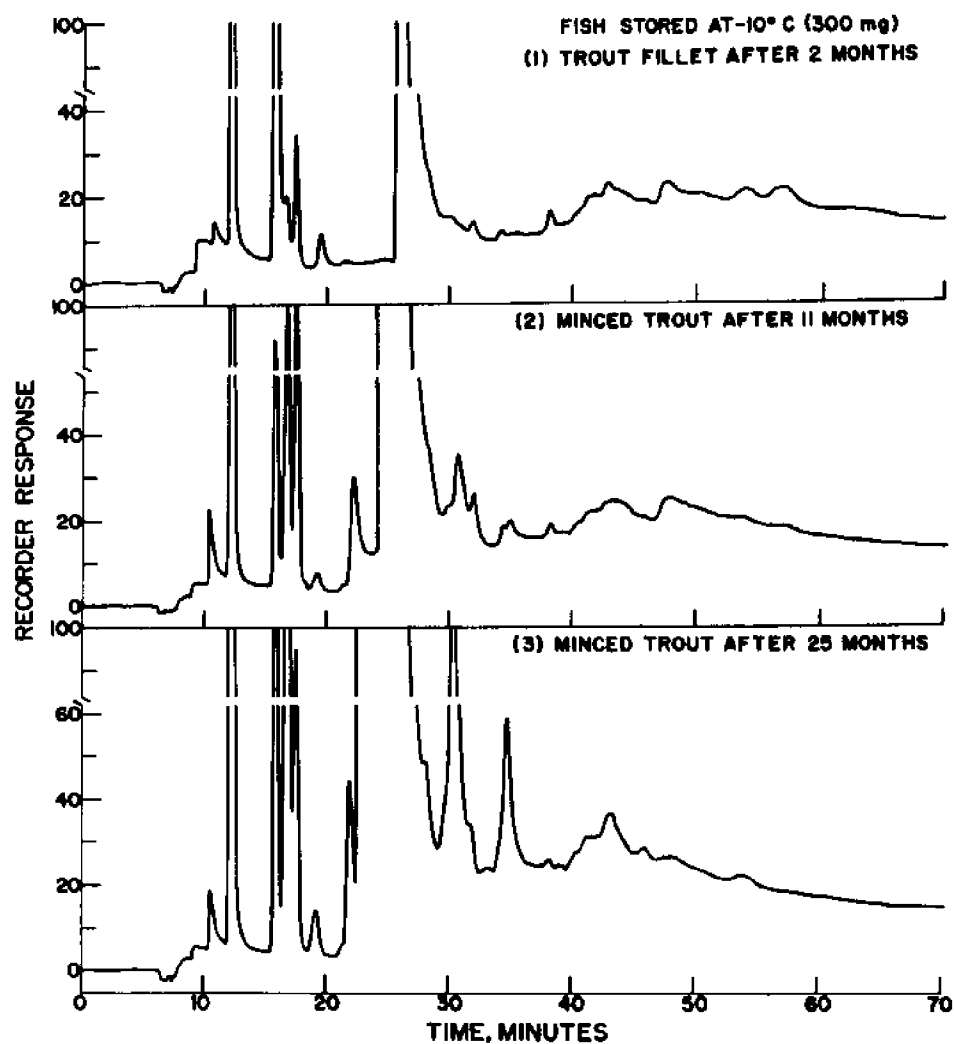Figure 1. Profile of volatiles obtained from aqueous solution of oysters stored under different conditions.

Figure 2. Profile of volatiles obtained from trout samples after
storage under refrigeration for different periods of time.

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DEVELOPMENT OF A PASTEURIZED OYSTER PRODUCT[1]

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INTRODUCTION

The College Park Laboratory (facility now located in Charleston, S.C.) of the Southeast Fisheries Center, NMFS, has been investigating the use of steam for the production of a pasteurized oyster that is an intermediate of the raw and commercially sterilized product. Our objective is to produce a lightly heated product that has high microbiological safety and quality with acceptable yields and organoleptic characteristics.

The primary criterion for oyster pasteurization was that the process would provide conditions sufficient for destruction of organisms of public health concern. To accomplish this, we established times and temperatures of exposure of shell stock that would destroy heat-resistant inoculated _Salmonella_ as indicators of destruction of other microorganisms in shell stock has been presented at other meetings and is now in press. Those experiments will not be discussed here except to say that the target temperatures for the pilot production of steamed-in-shell oysters were based on the conditions previously determined necessary to destroy these public health and other spoilage microorganisms. It is important to stress that the term pasteurization is used advisedly, since we are in the process of investigating any possibility of potential problems due to anaerobic sporeformers such as _Clostridium botulinum_ in lightly heat-treated oysters.

In the development of a "pasteurized" oyster, we began with shell stock rather than shucked oysters. It was felt that heating the shell stock would make shucking easier and also sanitize shell surfaces, which can be a source of contamination.

Atmospheric pressure steam was chosen for pasteurization after evaluation of several criteria that included consideration of:

1) Initial cost
2) Operating cost

1] Contribution Number 78-39C
2] Presently located at NMFS, Safety, Quality and Inspection Div. Washington, D.C.

3) Availability of equipment
4) Familiarity with process
5) Process effect on product quality
6) Recovery of bi-products

Among the methods considered were: hot water immersion, use of dry heat, microwave heating, and elevated and atmospheric pressure steaming.

Hot water immersion generates large volume of waste water. Direct water exposure during heating can cause leaching of soluble protein and flavor components. In addition, recovery of oyster liquor from large quantities of water is impractical.

Dry air heating has the obvious drawback of potential dehydration of the product during heating.

Microwave heating could be used for pasteurization. There is a rather high initial equipment cost, however, and it has been reported that microwave treatment can produce undesirable flavor changes in food.

When using elevated pressure steam for pasteurizing shell stock, significant problems were encountered in loading retorts, resulting in non-uniform heating.

The use of non-pressurized steam for oyster pasteurization has a relatively low initial operating cost. The equipment is readily available and easy to operate. The process is a familiar one with much information available on the effects of steam processing on various foods. Also, the liquor expressed during pasteurization could be recovered.

METHODS

Single layers of oysters were steamed on trays in a cabinet. The temperature was monitored using thermocouples inserted into the oyster belly masses through holes drilled into the shells. Single layers of oysters were used because stacking caused unacceptable disparity in heating. In addition, the oysters were separated into batches by weight so time/temperature variations due to size could be minimized. The cabinet used was a converted 12 cu. ft. upright freezer with sanitary steam provided by a small generator. Steam was distributed through a multi-holed sparger. As soon as the average internal temperature of the oysters reached the desired level, the steam was removed and the oysters were cooled with sprayed water to 30°C. They were then shucked and the yields determined.

Further tests included total counts on Standard Methods Agar and coliform determinations using standard MPN's and Violet Red Bile Agar. Shear press measurements were performed using a Kramer Shear Press equipped with a 1500 lb ring. Organoleptic evaluations were performed using triangulation taste panel testing with at least twelve panelists.

RESULTS

Table 1 shows the reduction in total plate count and coliforms as a function of temperature at the come-up time. The temperatures we chose to test were those for which the thermal death times of heat resistant bacteria had been established in previous investigations. At 60°C (140°F) the total count was sharply reduced and the coliforms were virtually eliminated. The remaining total aerobic count was due to <u>Bacillus</u>. No aerobic spoilage or public health significant organisms could be detected. It appears, therefore, that steaming oysters to 60°C or slightly above may be adequate for pasteurization.

Table 2 shows the yield of shucked meats compared to shell stock after heating. At 60°C there is approximately a 20% yield loss compared to the raw product whereas there is a 70% yield loss of the fully cooked product. The raw and pasteurized products are visibly similar with the exception that the pasteurized oysters are more uniform in color.

Once the initial microbiological criteria for pasteurization were satisfied, the food technological aspects of pasteurization of oysters were evaluated. Raw and pasteurized products were offered to taste panelists in order to ascertain differences and preferences. Acceptability was satisfactory. At a recent test comparing raw and pasteurized oysters, which were deep fried, the majority of the panelists could not differentiate between the two products. Of those who could tell the difference, preference was equally divided. We also had a workshop at our Laboratory in College Park to demonstrate the product to local industry members, who commented favorably on the product and are considering similar processing techniques.

To determine factors that influence taste panelist's judgements, we used a shear press to investigate the texture of the pasteurized product. To our knowledge, this type of work has not been done with oysters. The results of shear tests of raw, pasteurized and cooked oysters with and without the adductor muscle are compared in Table 3. Without the muscle the changes due to heating are relatively samll. However, when the muscle is present the shear press values increase sharply. This data supports the observations of taste panelists who noted increased firmness in the texture of the adductor muscle after heating. It appears, therfore, that the shear press can be a useful tool for measuring the texture of oyster products.

The "pasteurized" product can be economically produced with slightly modified equipment currently available in the industry. Table 4 shows the economic information for this product based on 14 and 50 bushel per day capacity pilot apparatus. These are incremental costs—that is, above the cost of the raw product. The labor cost per bushel for the larger production drops due to use of the same people for raw and pasteurizing operations and the fact that manipulations for both 14 and 50 bushel quantities are not significantly different.

CONCLUSIONS

A lightly heat-treated oyster product has been developed as an alternative to the raw product. Its closer similarity to raw oysters than to commercially sterile products indicates that it may find use as an adjunct to the raw project. It is also likely, with the sharp decrease in microbial load and the destruction of oyster enzymes at pasteurization temperatures, that shelf life could be extended beyond that for the raw product. It was also noted in our studies that steaming reduces undesirable coloration occasionally present in raw oysters. Last, but not least, application of steam for pasteurization of shell oysters facilitates shucking. Table 5 summarizes the product characteristics.

Future studies with pasteurized oysters will include further comparisons of shear press measurements (using a shear compressions cell to measure elasticity), taste panelist's evaluations, and continuation of work on the significance of anaerobic sporeformers in shell stock that may affect the safety and quality of heat-treated products. Long-term storage studies at 5°C and at freezer temperature (-40°C) have begun to evaluate product stability with reference to bacterial, organoleptic, and chemical characteristics.

Table 1. Effect of Steaming on Survival of bacteria in shell stock

Internal Temperature	Come-up Time (min)	TPC		Coliforms	
		Before Steam	After Steam	Before Steam	After Steam
56C(133F)	4.8	62,000	900	910	30
60C(140F)	5.5	47,000	230	1700	2
65C(149F)	6.0	55,000	29	340	< 1
71C(160F)	7.0	31,000	17	420	< 1
84C(180F)	12.0	51,000	15	1300	< 1

Table 2. Effect of steaming on the yield of shucked oysters.

Treatment	Yield	
	From shell stock	From raw shucked
Raw	12 - 15%	100%
Heated to:		
60°C	9.5 - 11%	80%
71°C	7.5 - 9.5%	65%
84°C	5.0 - 6.0%	40%
Autoclave	4.5%	30%

(Comparable to commercially
sterilized product)

Table 3. Effect of steaming on oyster texture.

Treatment	Internal Temperature (°C)	Shear Values (lbs/sq.in.)
Raw w muscle	18	172
w/o		144
Past. w muscle	62	388
w/o		196
Cooked w muscle	84	493
w/o		206

Table 4. Estimated incremental cost of pasteurization.

Capacity	14 bu/day		50 bu/day	
Initial Equipment Cost	$500		$2000	
	$/bu	$/day	$/bu	$/day
Equipment	0.15	2.08	0.17	8.33
Labor	1.72	24.00	0.48	24.00
Utilities	0.22	3.08	0.19	9.50
Total	$2.09	$29.16	$0.84	$42.23

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Table 5. Summary of product characteristics.

Raw	Pasteurized
2 week shelf life	3 week shelf life*
Public health organisms possible present	Eliminates coliforms and related organisms
Spoilage bacteria increase in 2 weeks	Bacteria essentially 0
Taste panel acceptable 2 weeks	Taste panel acceptable 2 weeks + (still under investigation)
Require skilled shucker	Shucking facilitated
May have undesirable coloration	Uniform color
--	0.30/lb. for 14 bu/day 0.12/lb. for 50 bu/day (Cost add'n to raw)

*possibly longer depending on results of future taste panels and chemical analyses.